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**WO 02/083606 A1**

(54) Title: A LINKER SYSTEM FOR THE SYNTHESIS AND SCREENING OF COMBINATORIAL LIBRARIES OF POLYAMINE DERIVATIVES ON WATER COMPATIBLE SUPPORTS

(57) Abstract: The invention is directed to resin-conjugated polyamine combinatorial libraries and methods for making and using them, e.g., methods for screening for polyamine binding molecules in biological samples. The invention provides protected benzophenone-substituted resins, and methods for their synthesis. The invention provides hydroxy-triarylmethane-conjugated resins, and methods for their synthesis. The invention provides chlorotriarylmethane resins, and methods for their synthesis. The invention provides resin-conjugated peptide libraries, and methods for their synthesis.

**TITLE**

A linker system for the synthesis and screening of combinatorial libraries of polyamine derivatives on water compatible supports

**TECHNICAL FIELD**

This invention generally relates the fields of chemistry and pharmaceutical drug preparation, particularly drug screening. In particular, the invention is directed to resin-conjugated polyamine combinatorial libraries, e.g., large libraries of unnatural polyamines, and methods for making and using them, e.g., methods for screening for polyamine binding molecules in biological samples.

**BACKGROUND**

Polyamines, exemplified in particular by spermine and spermidine, are ubiquitous biomolecules of prime importance in living systems. In fact, polyamines constitute one of very few classes of small organic compounds capable of interacting with the three natural biopolymers; polypeptides and proteins, nucleic acids, and oligosaccharides (see, e.g., Cohen, S.S. A Guide to the Polyamines, Oxford University Press: New York, 1998; Eliseev (1994) J. Am. Chem. Soc. 116:6081-6088). They are involved in a variety of biological processes dependent on the condensation and structural stabilization of DNA and RNA via electrostatic interactions with phosphate anions (see, e.g., Blagbrough (2000) J. Tetrahedron 56:3439-3447; Frydman (1996) J. Org. Chem. 61:2588-2589; Olive (1998) Biochemistry 37:6476-6484; Hamachi (1999) Bioorg. Med. Chem. Lett. 9:11215-1218). Biogenic polyamines also have an essential role in cell growth and differentiation (see, e.g., Tabor (1984) Ann. Rev. Biochem. 53:749-790). Various analogues have been evaluated as potential drugs against cancer; tumour cell growth has been observed to be accompanied by unusually high levels of biogenic polyamine concentrations, thus, the development of polyamine-based inhibitors for the anabolic enzymes has been considered as a potential approach for anticancer therapy (see, e.g., Marton (1995) Ann. Rev. Pharmacol. Toxicol. 35:55-91). Various analogues of biogenic polyamines have been evaluated as gene delivery agents (see, e.g., Garrett (2000) Bioorg. Med. Chem. 8:1779-1797). Several insects employ polyamine-based compounds as venom constituents for paralyzing preys or predators (see, e.g., Schulz (1997) Chem. Int. Ed. Engl. 36:314-326). One such example is

philanthotoxin-433, produced by the digger wasp Philanthus triangulum (see,e.g., Eldefrawi (1988) Proc. Natl. Acad. Sci. USA 85:4910-4913) and known to inhibit signal transmission in the central nervous system of mammals (see, e.g., Bähring (1998) Physiol. 509:635-650). In addition, acyclic and macrocyclic oligoamines such as cyclen are useful as artificial receptors (see, e.g., Izatt (1995) Chem. Rev. 95:2529-2586) and as ligands for organometallic reagents and catalysts in organic synthesis (see, e.g., Matsuo (1999) S. Org. Lett. 1:345-347).

The importance of polyamines combined with their limited availability from natural or commercial sources have generated significant interest in developing new and efficient methods to synthesize a wide variety of unnatural polyamines (see, e.g., Kuksa (2000) Synthesis 1189:1207).

**SUMMARY OF THE INVENTION**

The invention provides a method for the synthesis of a protected benzophenone-substituted resin **27** comprising the following steps: (a) providing a haloalkyl-terminated resin having a formula Resin-$(CH_2)_y$X, wherein y is an integer between 1 and 20 and X is a halogen; (b) providing a halo-magnesium-conjugated benzophenone derivative having a formula X-Mg-$(C_6H_4)$-$C(OR)_2$-Ph, wherein R is an alkyl substituent, X is a halogen, and $C_6H_4$ is a disubstituted benzene derivative; and, (c) reacting the haloalkyl-terminated resin of step (a) with the halo-magnesium-conjugated benzophenone derivative of step (b) under conditions comprising a CuBr salt, a CuI salt, or equivalent, and an anhydrous solvent, thereby producing a protected benzophenone-substituted resin having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-$C(OR)_2$-Ph, wherein y is an integer between 2 and about 25, or more. In one aspect the reaction temperature can be 70°C. In one aspect, the anhydrous solvent comprises a mixture of THF and HMPA (hexamethylphosphoramide), such as a solvent comprising a 1:5 mixture of hexamethylphosphoramide and THF.

In one aspect of the method, the haloalkyl-terminated resin comprises a polystyrene (PS) end-functionalized with a haloalkyl-terminated hydrophilic polyethylene glycol polymer having a formula PS-$(CH_2)_m$-$(OCH_2CH_2)_n$-$O(CH_2)_y$-X, wherein X is a halogen, n is an integer between 1 and about 100, m is 1 to about 20, and y is an integer between 1 and about 25, or more. In one aspect, n is an integer

larger than 100, e.g., 110, 120, 130, or longer. The integers m and y can be longer than 20 and 25, respectively.

In one aspect, the haloalkyl-terminated resin can comprise any PEG-grafted polystyrene, such as Tentagel™ or Argogel™. The hydrophilic polyethylene glycol polymer can be branched. The haloalkyl-terminated resin can comprise a polyoxyethylene-polyoxypropylene (MOEPOP) or a polyoxyethylene-polyoxypropylene (POEPOP) or a polyoxyethylene-polyoxetane (SPOCC).

In alternative aspects, the polystyrene resin comprises about 1% to about 2% crosslinked divinylbenzene. The haloalkyl-terminated resin can comprise a beaded diameter between about 10 microns and about 500 microns. The halo-magnesium-conjugated benzophenone can comprise a magnesium bromide-conjugated protected benzophenone. The halo-magnesium-conjugated benzophenone can comprise a protected 4-X-Mg-benzophenone, wherein X is Cl, Br or I.

In one aspect, the reactions conditions of step (c) can comprise stirring for about one to two, three or four days, or more. The reactions conditions of step (c) can further comprise quenching with a neutral or mildly acidic solution, such as a solution comprising an ammonium salt, or equivalent. In one aspect, the quenching solution is an aqueous saturated ammonium chloride solution.

The invention provides a protected benzophenone-substituted resin **27** produced by a method comprising the following steps: (a) providing a haloalkyl-terminated resin having a formula Resin-$(CH_2)_y$(X), wherein y is an integer between 1 and 20 and X is a halogen; (b) providing a halo-magnesium-conjugated benzophenone derivative having a formula X-Mg-$(C_6H_4)$-C$(OR)_2$-Ph, wherein R is an alkyl substituent, the ketone group is protected as an acetal group, X is a halogen; and, (c) reacting the haloalkyl-terminated resin of step (a) with the halo-magnesium-conjugated benzophenone of step (b) under conditions comprising a CuBr salt, or equivalent, and an anhydrous solvent, thereby producing a benzophenone-substituted resin **27** having a formula Resin-$(CH_2)_y$-$(C_6H_4)$- C$(OR)_2$-Ph, wherein y is an integer between 1 and about 25, or more.

The invention provides a protected benzophenone-substituted resin [3, Figure 14] having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-$C(OR)_2$-Ph, wherein y is an integer between 1 and about 25, or greater.

The invention provides a method for the synthesis of a deprotected benzophenone **28** having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-CO-Ph comprising the following steps: (a) providing a haloalkyl-terminated resin having a formula resin-$(CH_2)_y$X, wherein y is an integer between 1 and about 25 and X is a halogen; (b) providing a halo-magnesium-conjugated benzophenone derivative having a formula X-Mg-$(C_6H_4)$-$C(OR)_2$-Ph, wherein R is an alkyl substituent, the ketone group is protected as an acetal group, X is a halogen; and, (c) reacting the haloalkyl-terminated resin of step (a) with the halo-magnesium-conjugated benzophenone of step (b) under conditions comprising a CuBr salt, or equivalent, and an anhydrous solvent, thereby producing a benzophenone-substituted resin **27** having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-$C(OR)_2$-Ph, wherein y is an integer between 1 and about 25; (d) reacting the benzophenone-substituted resin of step (c) with an aqueous solution of a strong acid, examples of which are $HClO_4$, HCL, HBr, or an equivalent acid, $HClO_4$ being preferred, and $CH_2Cl_2$, or an equivalent; and (e) rinsing and drying the resin, thereby producing an free benzophenone **28** having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-CO-Ph, wherein y is an integer between 1 and about 25, or more.

In one aspect, in step (d), the solution used is a 1:10 mixture of 70% aqueous solution of perchloric acid ($HClO_4$) and methylene chloride. The reaction of step (d) can comprise mixing the beads at about room temperature. The reaction of step (d) can be between about 2 to about 24 hours, or more, e.g., for about 5, 10, 15, 20 or 25 hours. In one aspect, in step (e), the beads are rinsed with $H_2O$/THF (1:1), or equivalent, DMF/$Et_3N$ (1:3), or equivalent, MeOH, or equivalent, and $CH_2Cl_2$, or equivalent. In step (e) the beads can be rinsed several times.

The invention provides a method for the synthesis of a deprotected benzophenone **28** having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-CO-Ph comprising the following steps: (a) providing a protected benzophenone-substituted resin **27** having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-$C(OR)_2$-Ph, wherein y is an integer between 1 and about 25; (b) reacting the benzophenone-substituted resin of step (c) with an aqueous

solution of a strong acid, examples of which are $HClO_4$, HCl, HBr or an equivalent acid, $HClO_4$, being preferred and $CH_2Cl_2$, or an equivalent; and (c) rinsing and drying the resin, thereby producing a deprotected benzophenone **28** having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-CO-Ph, wherein y is an integer between 1 and about 25, or more.

The invention provides a deprotected benzophenone **28** having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-CO-Ph made by a method comprising the following steps: (a) providing a protected benzophenone-substituted resin **27** having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-$C(OR)_2$-Ph, wherein y is an integer between 1 and about 25, or more; (b) reacting the benzophenone-substituted resin of step (a) with an aqueous solution of a strong acid, examples of which are $HClO_4$, HCL, HBr, or an equivalent acid, $HClO_4$ being preferred, and $CH_2Cl_2$, or an equivalent; and (c) rinsing and drying the resin, thereby producing a deprotected benzophenone **28** having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-CO-Ph.

The invention provides a deprotected benzophenone **28** having a formula Resin-$(CH_2)_y$ -$(C_6H_4)$-CO-Ph, wherein y is an integer between 1 and about 25, or more.

The invention provides a method for the synthesis of a hydroxy-triarylmethane-conjugated resin **29** comprising the following steps: (a) providing a haloalkylterminated resin having a formula resin-$(CH_2)_y$ (X), wherein y is an integer between 1 and about 25, or more, and X is a halogen; (b) providing a halo-magnesium-conjugated benzophenone having a formula X-Mg-$(C_6H_4)$-$C(OR)_2$-Ph, wherein R is an alkyl substituent, the ketone group is protected as an acetal group, X is a halogen; (c) reacting the haloalkyl-terminated resin of step (a) with the halo-magnesium-conjugated benzophenone of step (b) under conditions comprising a CuBr salt, or equivalent, and an anhydrous solvent, thereby producing a benzophenone-substituted resin **27** having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-$C(OR)_2$-Ph, wherein y is an integer between 1 and about 25, or more; (d) reacting the benzophenone-substituted resin of step (c) with an aqueous solution of $HClO_4$, or equivalent acid, and $CH_2Cl_2$, or equivalent; (e) rinsing and drying the resin, thereby producing a deprotected benzophenone **28** having a formula Resin-$(CH_2)_y$ -$(C_6H_4)$-CO-Ph, wherein y is an integer between 1 and about 25, or more; (f) mixing a suspension of the resin

produced in step (e) **28** with a solution comprising an arylmagnesium halide, or equivalent; and, (g) adding an acidic solution, thereby producing a triarylmethane-conjugated resin **29** having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-C(OH)(Ar)-Ph, wherein y is an integer between 1 and about 25, or more.

In one aspect, the acidic solution of step (g) is a dilute aqueous solution of HCl, such as a 0.5M HCl solution. The method can further comprise washing and drying the hydroxy-triarylmethane-conjugated resin **29** produced in step (g). The washing conditions can comprise washing with a solution comprising $H_2O$, an aqueous solution of $NaHCO_3$, or equivalent, $H_2O$/THF (1:1), or equivalent, MeOH and $CH_2Cl_2$, or equivalent. The drying conditions can comprise high vacuum for greater than 12 hours in a dessicator or a glass vessel containing a drying agent, such as phosphorus pentoxide ($P_2O_5$). In step (f), the arylmagnesium halide can comprise a phenylmagnesium halide, or equivalent. In step (f), the arylmagnesium halide can be a functionalized biarylmagnesium halide having a formula XMg-$C_6H_4$-Y-$C_6H_4$-X, where Y is a selectively cleavable functionality, and X is a halide.

In one aspect, the selectively cleavable functionality is selected from the group consisting of $CH_2OCH_2$, S, $CH_2SCH_2$, Se, and Si, $CH_2SeCH_2$, $CH_2Si(Me)_2CH_2$, $SeCH_2$, $SCH_2$, $Si(Me)_2CH_2$, $CH_2Se$, $CH_2Si(Me)_2$, $CH_2S$ and equivalents The group also includes compounds wherein all the sulphurs are in their corresponding oxidized forms (e.g., SO and $SO_2$).

In one aspect, the hydroxy-triarylmethane-conjugated resin has a formula Resin-$(CH_2)_y$-$C_6H_4$-C(OH)($C_6H_4$-Y-$C_6H_4$-X)-Ph, wherein y is an integer between 1 and about 25, or more. In step (f), the arylmagnesium halide can be added dropwise under conditions comprising about room temperature.

The invention provides a method for making a hydroxy-triarylmethane-conjugated resin **29** comprising the following steps: (a) providing a deprotected benzophenone **28** having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-CO-Ph; (b) mixing a suspension of the resin of step (a) with a solution comprising an arylmagnesium halide, or equivalent; and, (c) adding an acidic solution, thereby producing a hydroxy-triarylmethane-conjugated resin **29** having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-C(OH)(Ar)-

Ph, wherein y is an integer between 1 and about 25, or more. In one aspect, the acidic solution is a dilute aqueous solution of HCl, such as a 0.5M HCl solution.

The invention provides a hydroxy-triarylmethane-conjugated resin **29** made by a method comprising the following steps: (a) providing a deprotected benzophenone **28** having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-CO-Ph; (b) mixing a suspension of the resin of step (a) with a solution comprising an arylmagnesium halide, or equivalent; and, (c) adding an acidic solution, thereby producing a triarylmethane -conjugated resin **29** having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-C(OH)(Ar)-Ph, wherein y is an integer between 1 and about 25, or more.

The invention provides a hydroxy-triarylmethane -conjugated resin **29** having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-C(OH)(Ar)-Ph, wherein y is an integer between 1 and about 25, or more.

The invention provides a method for the synthesis of a chlorotriarylmethane-conjugated resin 30 comprising the following steps: (a) providing a haloalkyl-terminated resin having a formula resin-$(CH_2)_y$X, wherein y is an integer between 1 and 20 and X is a halogen; (b) providing a halo-magnesium-conjugated benzophenone having a formula X-Mg-$(C_6H_4)$-$C(OR)_2$-Ph, wherein R is an alkyl substituent, the ketone group is protected as an acetal group, X is a halogen; and, (c) reacting the haloalkyl-terminated resin of step (a) with the halo-magnesium-conjugated benzophenone of step (b) under conditions comprising a CuBr salt, or equivalent, and an anhydrous solvent, thereby producing a benzophenone-substituted resin [3, Figure 14] having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-$C(OR)_2$-Ph, wherein y is an integer between 1 and about 25, or more; (d) reacting the benzophenone-substituted resin of step (c) with an aqueous solution of a strong acid, examples of which are $HClO_4$, HCL, HBr, or equivalent acid, $HClO_4$ being preferred, and $CH_2Cl_2$, or equivalent; (e) rinsing and drying the resin, thereby producing a deprotected benzophenone [4, Figure 14] having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-CO-Ph, wherein y is an integer between 1 and about 25, or more; (f) mixing a suspension of the resin produced in step (e) **28** with a solution comprising an arylmagnesium halide, or equivalent; (g) adding an acidic solution, thereby producing a hydroxy-triarylmethane -conjugated resin having a formula Resin-$(CH_2)_y$ -$(C_6H_4)$-C(OH)(Ar)-Ph, wherein y is

an integer between 1 and about 25, or more; (h) swelling the hydroxy-triarylmethane-conjugated resin of step (g) in dry $CH_2Cl_2$, or equivalent; and, (i) mixing the swelled hydroxy-triarylmethane-conjugated of step (h) with a solution comprising a $SOCl_2$ or an acetyl chloride or equivalent, thereby producing a chlorotriarylmethane-conjugated resin having a formula Resin-$(CH_2)_y$ -$(C_6H_4)$-C(Cl)-(Ar)-Ph, wherein y is an integer between 1 and 25, or more.

In one aspect, the method further comprises drying the chlorotriarylmethane-conjugated resin of step (i) under vacuum.

The invention also provides a method for the synthesis of a chlorotriarylmethane resin having a formula Resin-$(CH_2)_y$ -$(C_6H_4)$-C(Cl)(Ar)-Ph, wherein y is an integer between 1 and about 25, or more, comprising the following steps: (a) providing a hydroxy-triarylmethane-conjugated resin having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-C(OH)(Ar)-Ph, wherein y is an integer between 1 and about 25, or more; (b) swelling the hydroxy-triarylmethane **29** in dry $CH_2Cl_2$, or equivalent; and, (c) mixing the swelled resin of step (b) with a solution comprising a $SOCl_2$, an acetyl chloride or an equivalent, thereby producing a chlorotriarylmethane resin having a formula Resin-$(CH_2)_y$ -$(C_6H_4)$-C(Cl)(Ar)-Ph, wherein y is an integer between 1 and about 25, or more. TMSCl may replace $SOCl_2$, but it is not as effective.

The invention provides a chlorotriarylmethane resin having a formula Resin-$(CH_2)_y$ -$(C_6H_4)$-C(Cl)(Ar)-Ph made by a method comprising the following steps: (a) providing a hydroxy-triarylmethane-conjugated resin having a formula Resin-$(CH_2)_y$ -$(C_6H_4)$-C(OH)(Ar)-Ph, wherein y is an integer between 1 and about 25, or more; (b) swelling the hydroxy-triarylmethane resin in dry $CH_2Cl_2$, or equivalent; and, (c) mixing the swelled resin of step (b) with a solution comprising a $SOCl_2$, or equivalent, thereby producing a chlorotriarylmethane resin having a formula Resin-$(CH_2)_y$ -$(C_6H_4)$-C(Cl)(Ar)-Ph, wherein y is an integer between 1 and about 25, or more. There are alternatives to $SOCl_2$, e.g. TMSCl. However, $SOCl_2$ is most effective.

The invention provides a chlorotriarylmethane resin having a formula Resin-$(CH_2)_y$ -$(C_6H_4)$-C(Cl)(Ar)-Ph, wherein y is an integer between 1 and about 25, or more. In one aspect, the chlorotriarylmethane resin comprises a trityl resin.

The invention provides a method of making a spacer-conjugated triarylmethane resin, wherein the spacer comprises the general formula -W-R-ZH, where W and Z are selected from the group consisting of O, S, and NH, and R is an alkyl or an oxyalkyl spacer or equivalent or oligomeric version thereof, comprising the following steps: (a) providing a chlorotriarylmethane resin having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-C(Cl)(Ar)(Ph), wherein y is an integer between 1 and about 25; and, (b) reacting the resin of step (a) with HW-R-ZH in a solvent, where W and Z are selected from the group consisting of O, S, and NH, and R is an alkyl or an oxyalkyl spacer or equivalent or oligomeric version thereof, and $CH_2Cl_2$, or equivalent, thereby making the spacer-conjugated triarylmethane resin. In step (b), the solvent can comprise a methylene chloride, a DMF, a THF, or equivalent. The resin can be reacted with a large excess of HW-R-ZH. The temperature can be between room temperature and about 100°C.

In one aspect, the -W-R-ZH is -W-$(CH_2)_k$ -[O-$(CH_2)_m$]$_n$-O-$(CH_2)_k$-ZH, wherein m and n are integers between about 0 and about 25, and k is an integer between about 2 and about 25, or more; HW-$(CH_2)_k$-[O-$(CH_2)_m$]$_n$-O-$(CH_2)_k$-ZH can be HW-$(CH_2)_2$-[O-$(CH_2)_2$]$_n$-O-$(CH_2)_2$-ZH. In one aspect, -W-R-ZH is –NH-$(CH_2)_3$-[O-$(CH_2)_2$]$_2$-O-$(CH_2)_3$-$NH_2$. In one aspect, $H_2N[(CH_2)_nO(CH_2)_n]_m NH_2$ can be $H_2N[(CH_2)_2O(CH_2)_2]_2 NH_2$. In one aspect, -W-R-ZH is - NH-$(CH_2)_k$ -[O-$(CH_2)_m$]$_n$-O-$(CH_2)_k$-$NH_2$.

In one aspect, - NH-$(CH_2)_k$ -[O-$(CH_2)_m$]$_n$-O-$(CH_2)_k$- $NH_2$ is –NH-$(CH_2)_3$-[O-$(CH_2)_2$]$_2$-O-$(CH_2)_3$-$NH_2$. In one aspect, the spacer-conjugated triarylmethane resin is selected from the group consisting of Resin-$(CH_2)_k$ -[O-$(CH_2)_m$]$_n$-O-$(CH_2)_k$-ZH, wherein m and n are integers between about 0 and about 25, and k is an integer between about 2 and about 25, or more.

The invention provides a method of making a triarylmethane resin-conjugated peptide library comprising the following steps: (a) providing a spacer-conjugated triarylmethane resin, wherein the spacer comprises the general formula -W-R-ZH, where W and Z are selected from the group consisting of O, S, and NH, and R is an alkyl or an oxyalkyl spacer or equivalent or oligomeric version thereof; (b) providing a plurality of 9-fluorenylmethoxycarbonyl (Fmoc) protected amino acid

samples, wherein an amino acid comprises the general formula $HO_2C$-R'-NHFmoc; (c) mixing each amino acid sample of step (b) with a spacer-conjugated triarylmethane resin of step (a) by a 9-fluorenylmethoxycarbonyl (Fmoc) procedure with a carbodiimide reagent, a hydroxybenzotriazole and a base, or, a coupling reagent and a base (d) releasing the terminal Fmoc group by stirring the resin of step (c) in an about 20 to 30% solution of piperidine, or equivalent, in a solvent selected from the group consisting of a dichloromethane, a DMF, a NMP, a THF and an equivalent, thereby making a deprotected resin; (e) pooling the contents of all individual vessels, mixing, and redividing into equal amounts in the same number of samples as in step (b); and, (f) repeat steps (c) to (e) until the peptide has the desired length, thereby making the resin-conjugated peptide library. Many other known coupling agents may be used, one example of which is HBTU: O-genzotriazole-1-yl-N,N,$N^1$,$N^1$-tetramethyluronium hexafluorophosphate and the base may be diisopropylethylamine.

In one aspect, the spacer-conjugated triarylmethane resin is Resin-$(CH_2)_k$-$[O-(CH_2)_m]_n$-O-$(CH_2)_k$-ZH, wherein m and n are integers between about 0 and about 25, and k is an integer between about 2 and about 25. In one aspect, each individual amino acid sample of step (b) is mixed with the resin in a separate vessel. In one aspect, each individual amino acid sample of step (b) comprises a mixture comprising about 90% to about 95%, about 98%, about 99% or 100% of an Fmoc-protected amino acid $HO_2C$-R'-NH-Fmoc and 0 to about 10% of corresponding acylated amino acid $HO_2C$-R'-NHCOR". In one aspect, the acetyl (COR") group is selected from the group consisting of $CH_3CO$-, butyryl- , PrCO-, benzoyl, formyl, 9-anthracenylcarbonyl and equivalents thereof. In one aspect, the mixing of step (c) is in a solvent is selected from the group consisting of DMF, NMP and equivalents thereof. In one aspect, the carbodiimide reagent of step (c) is selected from the group consisting of dicyclohexyldiimide, diisopropylcarbodiimide, and equivalents thereof. In one aspect, the base of step (c) is diisopropylethylamine, or equivalents thereof. In one aspect, the mixing of step (c) takes place for between about 2 and about 24 hours, or more, at room temperature. The coupling reagent of step (c) can be selected from the group consisting of PyBroP, HATU, PyBOP, HBTU and equivalents thereof. The method can further comprise capping the terminal amino group with a COR'" group by

reaction with the corresponding anhydride (R'''CO)$_2$O or halide R'''COX or equivalents. The COR'' capping group can be selected from the group consisting of acetyl ($COCH_3$), benzoyl (COPh), formyl (COH), propionyl (COEt), 9-anthracenyl-carbonyl and equivalents. The amino acids are selected from the group consisting of all natural amino acids, including both enantiomers, including, but not limited to, species such as L-Ala, D-Ala, L-Nva, D-Nva, L-Met, D-Met, L-Phe, D-Phe, L-Ser(tBu), D-Ser(tBu), L-Leu, D-Leu, L-Hfe, D-Hfe, L-Gin, D-Gin, L-Gln, D-Gln, L-Trp(Boc), D-Trp(Boc), 2-aminocyclohexane carboxylic acid (cis and trans), 4-aminocyclohexane carboxylic acid (cis and trans), all the isomers of aminomethyl-benzoic acids, $HO_2C$-R'-NHFmoc where R' = $(CH_2)_n$ where n is an integer between 1 to about 12 and where R'= all positional isomers of biphenylmethyl ($C_6H_4$-$C_6H_4$-$CH_2$-).

In one aspect, the peptide library comprises the general formula Resin-$(CH_2)$y-$(C_6H_4)$-C(Ar)(Ph)-W-R-Z-(CO-R'-NH)$_n$-COR''', wherein each unit "n" (i.e., -(CO-R'-NH) ) is terminated by 0 to about 10%, or more, COR'', wherein COR'' is selected from the group consisting of $CH_3CO$-, butyryl- , PrCO-, benzoyl, formyl and equivalents thereof, and COR''' is selected from the group consisting of acetyl ($COCH_3$), benzoyl (COPh), formyl (COH), propionyl (COEt), 9-anthracenylcarbonyl and equivalents.

In alternative aspects, the resin-conjugated peptide library is a resin-conjugated monoamide library, a resin-conjugated di-peptide library, a resin-conjugated tri-peptide library, and a resin-conjugated tetrapeptide library.

The invention provides a resin-conjugated peptide library made by a method comprising the following steps: (a) providing a spacer-conjugated triarylmethane resin, wherein the spacer comprises the general formula -W-R-ZH, where W and Z are selected from the group consisting of O, S, and NH, and R is an alkyl or an oxyalkyl spacer or equivalent or oligomeric version thereof; (b) providing a plurality of 9-fluorenylmethoxycarbonyl (Fmoc) protected amino acid samples, wherein an amino acid comprises the general formula $HO_2C$-R'-NHFmoc; (c) mixing each amino acid sample of step (b) with a spacer-conjugated triarylmethane resin of step (a) by a 9-fluorenylmethoxycarbonyl (Fmoc) procedure with a carbodiimide

reagent, a hydroxybenzotriazole and a base, or, a coupling reagent and a base, (d) releasing the terminal Fmoc group by stirring the resin of step (c) in an about 20 to 30% solution of piperidine, or equivalent, in a solvent selected from the group consisting of a dichloromethane, a NMP, a THF, a DMF and an equivalent, thereby making a deprotected resin; (e) pooling the contents of all individual vessels, mixing, and redividing into equal amounts in the same number of samples as in step (b); (f) repeat steps (c) to (e) until the peptide has the desired length, thereby making the resin-conjugated peptide library.

In one aspect, the resin-conjugated peptide library is selected from the group consisting of a resin-conjugated monoamide library, a resin-conjugated di-peptide library, a resin-conjugated tri-peptide library, a resin-conjugated tetrapeptide library, a resin-conjugated library wherein each member is five peptides in length, a resin-conjugated library wherein each member is six peptides in length, a resin-conjugated library wherein each member is seven peptides in length, a resin-conjugated library wherein each member is eight peptides in length, a resin-conjugated library wherein each member is nine peptides in length and a resin-conjugated library wherein each member is ten peptides in length.

The invention provides a method of making a resin-conjugated polyborane-amine adduct library comprising the following steps: (a) providing a spacer-conjugated triarylmethane resin, wherein the spacer comprises the general formula -W-R-ZH, where W and Z are selected from the group consisting of O, S, and NH, and R is an alkyl or an oxyalkyl spacer or equivalent or oligomeric version thereof; (b) providing a plurality of 9-fluorenylmethoxycarbonyl (Fmoc) protected amino acid samples, wherein an amino acid comprises the general formula $HO_2C$-R'-NHFmoc; (c) mixing each amino acid sample of step (b) with a spacer-conjugated triarylmethane resin of step (a) by a 9-fluorenylmethoxycarbonyl (Fmoc) procedure with a carbodiimide reagent, a hydroxybenzotriazole and a base, or, a coupling reagent and a base, (d) releasing the terminal Fmoc group by stirring the resin of step (c) in an about 20 to 30% solution of piperidine, or equivalent, in a solvent selected from the group consisting of a dichloromethane, a DMF, a NMP, a THF and equivalents, thereby making a deprotected resin; (e) pooling the contents of all individual vessels, mixing,

and redividing into equal amounts in the same number of samples as in step (b); (f) repeat steps (c) to (e) until the peptide has the desired length, thereby making a resin-conjugated peptide library; and, (g) reacting the resin-conjugated peptide library of step (f) with about 0.1 to about 1.0 M $BH_3$ (based on 10 ml solvent per gram/resin) or equivalent and THF or equivalent, thereby making a resin-conjugated polyborane-amine adduct library. Typically, the contents from step (e) should be pooled to start with. $BH_3$ can be between about 0.1 to about 1.0 M at approximately 1g resin/10 mL. Extensive resin washing with dry THF may be needed. In one aspect, the reaction of step (e) is done under conditions comprising a temperature of about 65°C. In one aspect, the reaction of step (e) is done under conditions comprising lasting for about 12 hours to about 5 days, or more.

The invention provides a resin-conjugated polyborane-amine adduct library made by a method comprising the following steps: (a) providing a spacer-conjugated triarylmethane resin, wherein the spacer comprises the general formula -W-R-ZH, where W and Z are selected from the group consisting of O, S, and NH, and R is an alkyl or an oxyalkyl spacer or equivalent or oligomeric version thereof; (b) providing a plurality of 9-fluorenylmethoxycarbonyl (Fmoc) protected amino acid samples, wherein an amino acid comprises the general formula $HO_2C$-R'-NHFmoc; (c) mixing each amino acid sample of step (b) with a spacer-conjugated triarylmethane resin of step (a) by a 9-fluorenylmethoxycarbonyl (Fmoc) procedure with a carbodiimide reagent, a hydroxybenzotriazole and a base, or, a coupling reagent and a base, (d) releasing the terminal Fmoc group by stirring the resin of step (c) in an about 20 to 30% solution of piperidine, or equivalent, in a solvent selected from the group consisting of a dichloromethane, a DMF, a NMP, a THF and equivalents, thereby making a deprotected resin; (e) pooling the contents of all individual vessels, mixing, and redividing into equal amounts in the same number of samples as in step (b); (f) repeat steps (c) to (e) until the peptide has the desired length, thereby making the resin-conjugated peptide library; and, (g) reacting the resin-conjugated peptide library of step (f) with about 0.1 to about 1.0 M $BH_3$ (based on 10 ml solvent per gram/resin) or equivalent and THF or equivalent, thereby making a resin-conjugated polyborane-amine adduct library.

The invention provides a resin-conjugated polyborane-amine adduct library comprising a spacer-conjugated triarylmethane resin, wherein the spacer is selected from the group consisting of $-HN[(CH_2)_nO(CH_2)_n]_m NH_2$, wherein n is an integer between 1 and about 5 and m is an integer between 1 and about 25, or more.

The invention provides a method of making a resin-conjugated polyamine library comprising the following steps: (a) providing a spacer-conjugated triarylmethane resin, wherein the spacer comprises the general formula -W-R-ZH, where W and Z are selected from the group consisting of O, S, and NH, and R is an alkyl or an oxyalkyl spacer or equivalent or oligomeric version thereof; (b) providing a plurality of 9-fluorenylmethoxycarbonyl (Fmoc) protected amino acid samples, wherein an amino acid comprises the general formula $HO_2C$-R'-NHFmoc; (c) mixing each amino acid sample of step (b) with a spacer-conjugated triarylmethane resin of step (a) by a 9-fluorenylmethoxycarbonyl (Fmoc) procedure with a carbodiimide reagent, a hydroxybenzotriazole and a base, or, a coupling reagent and a base, (d) releasing the terminal Fmoc group by stirring the resin of step (c) in an about 20 to 30% solution of piperidine, or equivalent, in a solvent selected from the group consisting of a dichloromethane, a DMF, a NMP, a THF and equivalents, thereby making a deprotected resin; (e) pooling the contents of all individual vessels, mixing, and redividing into equal amounts in the same number of samples as in step (b); (f) repeat steps (c) to (e) until the peptide has the desired length, thereby making the resin-conjugated peptide library; (g) reacting the resin-conjugated peptide library of step (f) with about 0.1 to about 1.0 M $BH_3$ (based on 10 ml solvent per gram/resin) or equivalent and THF or equivalent, thereby making a resin-conjugated polyborane-amine adduct library; and, (h) reacting the resin-conjugated polyborane-amine adduct library of step (g) to oxidative conditions, thereby making a resin-conjugated polyamine library.

In one aspect, the oxidative conditions are mild oxidative conditions. In one aspect, these conditions comprise using excess iodine in THF. In one aspect, the mild oxidative conditions comprise mildly acidic conditions at about pH 5. In one aspect, the mildly acidic conditions comprise buffering by an acetic acid-trialkylamine buffer in a 2:1 volume ratio in THF, or equivalent. In one aspect, the oxidative

conditions of step (h) comprise a solution comprising $I_2$, AcOH, diisopropylethylamine (DIPEA), triethylamine, THF, or equivalents thereof; in one aspect, there is a reaction time of about 1 to about 6 hours, or more. In one aspect, the method further comprises reaction with a solution comprising an $Et_3N$, THF, DMF, or equivalents thereof. An alternative to the oxidative workup is Houghten's workup: shaking the resin, after borane reduction and usual washings, in a solution of neat piperidine at 65 degrees for 12-16 hours. The resin is then filtered at room temperature and then washed several times with the or equivalent usual solvents (DMF, MeOH, dichloromethane, NMP, THF and equivalent).

The invention provides a resin-conjugated polyamine library made by a method comprising the following steps: (a) providing a spacer-conjugated triarylmethane resin, wherein the spacer comprises the general formula -W-R-ZH, where W and Z are selected from the group consisting of O, S, and NH, and R is an alkyl or an oxyalkyl spacer or equivalent or oligomeric version thereof; (b) providing a plurality of 9-fluorenylmethoxycarbonyl (Fmoc) protected amino acid samples, wherein an amino acid comprises the general formula $HO_2C$-R'-NHFmoc; (c) mixing each amino acid sample of step (b) with a spacer-conjugated triarylmethane resin of step (a) by a 9-fluorenylmethoxycarbonyl (Fmoc) procedure with a carbodiimide reagent, a hydroxybenzotriazole and a base, or, a coupling reagent and a base, (d) releasing the terminal Fmoc group by stirring the resin of step (c) in an about 20 to 30% solution of piperidine, or equivalent, in a solvent selected from the group consisting of a dichloromethane, a DMF, a NMP, a THF and equivalents, thereby making a deprotected resin; (e) pooling the contents of all individual vessels, mixing, and redividing into equal amounts in the same number of samples as in step (b); (f) repeat steps (c) to (e) until the peptide has the desired length, thereby making the resin-conjugated peptide library; (g) reacting the resin-conjugated peptide library of step (f) with about 0.1 to about 1.0 M $BH_3$ (based on 10 ml solvent per gram/resin) or equivalent and THF or equivalent, thereby making a resin-conjugated polyborane-amine adduct library; and, (h) reacting the resin-conjugated polyborane-amine adduct library of step (g) to oxidative conditions, thereby making a resin-conjugated polyamine library. In one aspect, the polyamines comprise the general formula Z-(CO-

R'-NH)$_n$-COR''', wherein each unit n is terminated by 0 to about 10% COR", wherein COR" is selected from the group consisting of $CH_3CO$-, butyryl- , PrCO-, benzoyl, formyl and equivalents thereof, and COR''' is selected from the group consisting of acetyl ($COCH_3$), benzoyl (COPh), formyl (COH), propionyl (COEt), 9-anthracenylcarbonyl and equivalents.

The invention provides a resin-conjugated polyamine library comprising a spacer-conjugated triarylmethane resin, wherein the spacer is selected from the group consisting of $-HN[(CH_2)_nO(CH_2)_n]_m NH_2$, wherein n is an integer between 1 and about 5 and m is an integer between 1 and about 25, and the polyamine library comprises the general formula Resin-$(CH_2)_y$-$(C_6H_4)$-C(Ar)(Ph)-W-R-Z-(CO-R'-NH)$_n$-COR''', wherein each unit n is terminated by 0 to about 10% COR", wherein COR" is selected from the group consisting of $CH_3CO$-, butyryl- , PrCO-, benzoyl, formyl and equivalents thereof, and COR''' is selected from the group consisting of acetyl ($COCH_3$), benzoyl (COPh), formyl (COH), propionyl (COEt), 9-anthracenylcarbonyl and equivalents.

The invention provides a method for screening for a polyamine binding molecule in a biological sample comprising the following steps: (a) providing a biological sample; (b) providing a resin-conjugated polyamine library of the invention; (c) mixing the biological sample with the resin-conjugated polyamine library; and (d) washing the resin-conjugated polyamine library and determining if a biological molecule has specifically bound to a resin-conjugated polyamine. In one aspect, the beaded library and the sample are incubated in buffered water. In another aspect, screening is by using a biomolecule tagged with a flurorescent dye or a color dye. Beads containing polyamine ligands become either fluorescent or colored as visualized under a microscope.

In alternative aspects, the biological molecule comprises a nucleic acid, such as a DNA, a cDNA, an RNA, such as an mRNA, a lipid, a polypeptide or protein (including peptides, peptidomimetics, and the like), a mono- or polysaccharide. The biological sample can be mixed with the resin-conjugated polyamine library under conditions comprising buffered water.

The invention provides a method for screening for a polyamine binding molecule in a biological sample comprising the following steps: (a) providing a biological sample; (b) providing a resin-conjugated polyamine library of the invention, wherein the polyamine library is further derivatized to a tertiary polyamine library by alkylation with an alkyl or an aryl halide or further derivatized by reductive amination with aldehydes and a hydride reagent, or, further derivatized to an acylpolyamine library by reaction with acid anhydrides having the general formula $(R''''CO)_2O$ or halides having the general formula R''''COX or equivalents, wherein R is an alkyl or an aryl substituent, X is a halogen; (c) mixing the biological sample with the resin-conjugated polyamine library; and washing the resin-conjugated polyamine library and determining if a biological molecule has specifically bound to a resin-conjugated polyamine. In one aspect, the alkyl halide comprises a compound selected from the group consisting of ortho-bromomethylboronic acid anhydride or a corresponding boronic ester, allylbromide, benzyl bromide, methyl iodide, ethyl iodide, and equivalents thereof.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

All publications, patents, patent applications cited herein are hereby expressly incorporated by reference for all purposes.

**DESCRIPTION OF DRAWINGS**

Figure 1 is a schematic illustrating a mechanistic scheme including probable intermediates in the reduction of secondary amides by borane.

Figure 2 is a schematic illustrating the oxidative cleavage of resin-bound borane-amine adducts; as described in detail in Example 1, below.

Figure 3 is a schematic representing all four possible diastereomeric tetraamines containing a reduced Phe-Ala dipeptide unit made by the protocol described in detail in Example 1, below.

Figure 4 is a representation of the results of high pressure liquid chromatography (HPLC) analyses of the diastereomeric tetraamines shown in Figure 3:

Figure 4A: HPLC-ES-MS trace of LD-3; Figure 4B: HPLC-ES-MS trace of DD-3; Figure 4C: co-injection of LD-3 and DD-3; as described in detail in Example 1, below.

Figure 5 is a schematic illustrating the synthesis and treatment of amines by oxidative cleavage of resin-bound borane-amine adducts using a Rink resin linker; as described in detail in Example 1, below.

Figure 6 is a schematic illustrating the synthesis and treatment of a series of model dipeptides **6** by oxidative cleavage of resin-bound borane-amine adducts, as described in detail in Example 1, below.

Figure 7 is a schematic illustrating a procotol wherein oligo(*sec*-amines) are acylated to provide tertiary polyamide derivatives, as described in detail in Example 1, below.

Figure 8 is a schematic illustrating a protocol wherein an oligo(borane-amine) intermediate is reacted with excess propionaldehyde, as described in detail in Example 1, below.

Figure 9 is a schematic illustrating a protocol wherein a model tripeptide is reduced with a central tertiary amide, as described in detail in Example 1, below.

Figure 10(A) , is a schematic illustrating a protocol wherein *N*-acetyl amino acid ester is treated under reduction conditions, followed by a buffered iodine work-up, to obtain a high yield of crude *N*-alkylated product; and, Figure 10(B) is a schematic illustrating a quantitative determination of borane-amines using iodine in a sodium acetate/acetic acid aqueous buffer, as described in detail in Example 1, below.

Figure 11 is a schematic illustrating a protocol wherein borane-amine adducts are cleaved through the action of iodine, as described in detail in Example 1, below.

Figure 12 is a schematic illustrating a mechanistic pathway for borane-amine cleavage by iodine using an exemplary method of the invention, as described in detail in Example 1, below.

Figure 13 is a schematic illustrating an exemplary procedure for the borane reduction/iodine work-up of resin-bound polyamides, as described in detail in Example 1, below.

Figure 14 is a schematic illustrating exemplary steps in the preparation of a "chlorotriarylmethane conjugated PEG-PS resin" as a first step in the synthesis of libraries of "exo-peptides," or polyamines, of the invention using hydrophilic triarylmethyl resins, as described in detail in Example 2, below.

Figure 15A is a schematic illustrating an exemplary procedure for reduction of polyamide **31** to model polyamine **32** with the $BH_3/I_2$-method. Figure 15B is a schematic illustrating the structure of an exemplary "exopeptide" library comprising up to 10,368 rotamers, wherein n = 1 and m = 1 to 4. Figure 15C is a schematic illustrating two hypothetical "intermediate" or "byproduct" structures (Structure I and Structure II) that may be generated in the synthesis of libraries of "exo-peptides," or polyamines, of the invention, as described in detail in Example 2, below.

Figure 16 is a schematic illustrating exemplary steps in the preparation of the libraries of "exo-peptides," or polyamines, of the invention using hydrophilic triarylmethyl resins, as described in detail in Example 2, below.

Figure 17 is a schematic illustrating 18 amino acids were used as building blocks to make the initial resin-bound dipeptides library of **33** (Figure 16), as described in detail in Example 2, below.

Figure 18 is a schematic illustrating the 18 acyl protected amino acids used as encoding compounds to identify combinatorial library members, as described in detail in Example 2, below.

Figure 19 is a schematic illustrating four capping reagents, with larger differences in structure, were used at different stages of the combinatorial library synthesis process, as described in detail in Example 2, below.

Figure 20 is a schematic illustrating model exo-peptide compound **38** with three differently bulky substituents (benzyl, methyl, t-butyl) was synthesized for checking its possible rotamers, as described in detail in Example 2, below.

Figure 21 is a schematic illustrating the exemplary partial termination encoding method, as described in detail in Example 2, below.

Figure 22 illustrates the synthesis and screening of bead-supported unnatural polyamine libraries for multivalent ion-pairing, wherein $R^1$- $R^3$ represent structurally diverse spacers;

Figure 23 illustrates structures for model trisulfonated targets **1**: SPADNS and **2**: New Coccine;

Figure 24 illustrates structures for a set of structurally diverse amino acid building blocks for the generation of polyamine libraries, wherein 2Acc is employed as racemate, 4 Acc and 2 Acc are exclusively *cis*, 4Amc is exclusively of *trans* configuration;

Figure 25 illustrates possible conformations of dye targets 1 and 2 with indicated oxygen-to-oxygen distances ( ´ ) measured between remote sulfonate oxygens;

Figure 26 illustrates an HPLC chromatogram of a 1:1 mixture of dyes **1** and **2** (A), and after elution of bound material from resin-supported $-(CH_2)_{12}$-NH-2Acc$^R$-6Ahx$^R$-Et(B). Conditions: 5 to 20% (10 min.) acetonitrile in water containing 25 mM phosphate buffer (pH 7.0), U.V. detection at 350 nm. Note: dye 1 tends to elute under two forms using these conditions although it is homogeneous by NMR.

Like reference symbols in the various drawings indicate like elements.

**DETAILED DESCRIPTION**

The invention provides bead-supported oligoamine, e.g., polyamine, libraries and solid-phase synthetic methodologies for making and using them. The invention includes a novel oxidative work-up for the cleavage of borane-amine adducts from the reduction of solid-supported polyamides. The beaded solid supports comprise water-compatible triarylmethane (triarylmethyl) resins. The invention also provides various applications for these novel libraries of polyamine derivatives in chemical biology and combinatorial catalysis.

The libraries of the invention for the first time provide the capability to screen polyamines on a hydrophilic, water-compatible support, which allows for the screening of biomolecules. The invention provides a new trityl-type linker to a support that tolerates the chemistry needed to make the polyamine libraries and does not give premature release of the polyamine. This is crucial because commercially available hydrophilic supports with a trityl-based linker have failed to tolerate the chemistry to make polyamines from polypeptides. The split-pool synthesis described herein has the power to generate large polyamine libraries.

The invention provides methods for screening polyamine-binding molecules in a biological sample using the resin-conjugated polyamine libraries of the invention. Significantly, the invention provides libraries of unnatural polyamines. These polyamine derivatives are important targets for the screening of therapeutically useful molecules. Thus, the invention provides a powerful new tool for identification of polyamine-binding molecules. Because polyamines are known to act as neuropharmaceuticals, insecticides, and natural polyamines are known to play an essential roles in a variety of cell functions, including DNA replication, protein synthesis and gene expression (see discussion below), the isolation and identification of molecules that bind to and possible alter polyamine function has great potential in medicine and industry.

In making the large, bead-supported oligoamine (e.g., polyamine) libraries of the invention, exhaustive reduction of polypeptide precursors is used. The invention combines routine solid-phase methods with this novel approach to the reduction of polypeptide precursors (as opposed to a linear strategy, in which masked diamine building blocks are added one by one through sequential manipulations of nitrogen-containing functionalities, as described, e.g., by Kuksa (2000) supra; and, Fauchet (1994) C. Bioorg. Med. Chem. Lett. 4:2559-2562; Nash (1996) Tetrahedron Lett. 37:2625-2628; Byk (1997) D. Tetrahedron Lett. 38:3219-3222; Marsh (1997) Tetrahedron 53:17317-17334; Page (1998) Bioorg. Med. Chem. Lett. 8:1751-1756; Jefferson (2000) J. Comb. Chem. 2:441-444; as examples of solid-phase hemisyntheses of polyamine derivatives elaborated from an advanced polyamine template). Moreover, the invention's novel approach allows the inclusion of chiral side chains derived from alpha-amino acids.

The methods include a mild oxidative work-up protocol using iodine for the cleavage of borane-amine adducts arising from the borane-promoted reduction of polyamides supported onto triarylmethane (e.g., triphenylmethyl, or trityl) -based resins. In one embodiment, this procedure uses an acetic acid-acetate buffer solution. Chiral polyamines with diverse side chain functionalities can be generated as free bases without premature release from this solid support. There is essentially no racemization using this method.

Mechanistically, the reduction of secondary amides by diborane is believed to proceed as set forth in **Figure 1**. Hydrogen evolution is first observed as a result of an acid-base reaction between the first equivalent of borane and three amidic hydrogens. Then, the resulting intermediate I requires the addition of two hydride equivalents per amide unit in order to effect complete reduction leading to the triaminoborane intermediate II (on the basis of possible structural restraints, the existence of intramolecular trimeric species such as I and II is not obvious in the reduction of hindered or rigid polyamides; similarly, partial site isolation in solid-supported chemistry may alter the formation of such aggregates). In theory three hydride equivalents should be sufficient for the reduction of a secondary amide. It is known, however, that the formation of a stable borane-amine aminoborane adduct of type III occurs faster than the reduction of intermediate I. Under normal temperature conditions (less than 65°C), species III are not sufficiently active to further reduce I. Consequently, excess borane is required to ensure complete reduction of I such that six hydride equivalents per amide are required in the overall process. A number of protocols were developed in order to avoid the unnecessary waste of an extra mole of borane ultimately consumed in the formation of borane-amine adducts V. Brown (1981) Synthesis 996-997, proposed the use of boron trifluoride, a superior amine coordinating agent which is mutually compatible with borane, thus allowing the use of only one equivalent of the latter. Alternatively, Bonnat (1991) Synth. Comm. 21:1579, found that the use of elevated temperatures such as refluxing toluene (110 °C) makes the use of only three hydride equivalents possible. Presumably, the intermediate III initially formed is reductively active under these thermal conditions, thereby avoiding the addition of an extra equivalent of borane. The disproportionation of III to aminoborane IV and borane may occur, or perhaps a more plausible explanation is that the dissociation of III back to II and borane may become rapid enough to allow further reduction of leftover I.

Example 1, below, describes making triamine products (Compound 8, Figure 6) in high yields and good to excellent purity using a series of model oligomeric secondary diamides (, compound 6, in Example 1) containing various α-amino acid residues (e.g., Val, Phe, Tyr, Ser, Cys, Met, Gln, Trp). In another example (Figure 9),

a substrate containing a tertiary amide (compound 15), formed a rather unusual triaminoborane intermediate that required more stringent work-up conditions to liberate the polyamine product 20, Figure 9. The reduction of oligomeric tertiary amides, like compound 9, Figure 7 was found sluggish, but these compounds could nonetheless be obtained in high purity from in situ reductive amination of the corresponding secondary amines. Control studies, carried out in solution with model secondary amide, compound23, Figure 11, confirmed the efficiency of the buffered iodine solution and highlighted several advantages (including no need for heating, no need for strong bases or acids) over existing methods for the cleavage of borane-amine adducts.

In Example 1, a mechanism involving all buffer components (iodine, acetic acid, and acetate ion) is also described, in which borane-amine adducts are transformed first to the monoiodoborane-amine, then to the corresponding acetoxyborane-amine adduct of much weaker coordination affinity. The acetoxyborane-amine adduct dissociated readily and was trapped by the acetic acid to provide the desired secondary amine. This reduction/ oxidative work-up protocol is useful as a general method for the facile solid-phase synthesis of polyamines for eventual release in solution and use in various applications. It is also very useful toward the synthesis and screening of bead-supported libraries of free oligoamines assembled through split-pool methods.

Example 2 describes the preparation of combinatorial libraries of "exo-peptides" or polyamines using hydrophilic triarylmethyl resins. An exemplary "exo-peptide" library of the invention having with up to 10,368 rotamers was synthesized, demonstrating the power of the "split-pool" protocol incorporated in the methods of the invention.

## DEFINITIONS

Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs. As used herein, the following terms have the meanings ascribed to them unless specified otherwise.

As used herein, the term "alkyl" is used to refer to a branched or unbranched, saturated or unsaturated, monovalent hydrocarbon radical having from 1

to about 30 carbons, or, from about 4 to about 20 carbons, or, from about 6 to about 18 carbons. When the alkyl group has from 1 to about 6 carbon atoms, it can be referred to as a "lower alkyl." Suitable alkyl radicals include, for example, structures containing one or more methylene, methine and/or methyne groups. The term also includes branched structures have a branching motif similar to i-propyl, t-butyl, i-butyl, 2-ethylpropyl, etc. As used herein, the term encompasses "substituted alkyls." "Substituted alkyl" or "alkyl substituent" can also include one or more functional groups such as lower alkyl, aryl, acyl, halogen (*i.e.*, alkylhalos), hydroxy, amino, alkoxy, alkylamino, acylamino, thioamido, acyloxy, aryloxy, aryloxyalkyl, mercapto, thia, aza, oxo, both saturated and unsaturated cyclic hydrocarbons, heterocycles and the like. These groups may be attached to any carbon of the alkyl moiety. Additionally, these groups may be pendent from, or integral to, the alkyl chain. For example, "alkyl substituent" can include methyl, ethyl, ethylene or propylene (cyclic acetals).

The term "boronic acid" includes any form of boronic acid or equivalent, including any functionalized boronic acid, e.g., aryl boronic acids, such as such as phenylboronic acids; see also, U.S. Patent Nos. 6,083,903; 6,075,126; 6,037,490; 6,031,117; 6,013,783; 5,840,677; 5,780,454; 5,739,318, describing boronic acids that can be used in the compositions and methods of the invention, and how to make them. See also U.S. Patent Nos. 5,594,111, 5,623,055, 5,668,258, 5,648,470, 5,594,151, 5,668,257, 5,677,431, 5,688,928, 5,744,627, 5,777,148, 5,831,045 and 5,831,046; describing additional boronic acid reagents and boronic acid complexing reagents that can be used in the compositions and methods of the invention.

As used herein, the terms "mixing or "contacting" or "reacting" refer to the act of bringing components of a reaction into adequate proximity such that the reaction can occur. More particularly, as used herein, the terms "reacting," "mixing" and "contacting" can be used interchangeably with the following: combined with, added to, mixed with, passed over, flowed over, interacted with, etc.

The term "halogen" as used herein refers to fluorine, bromine, chlorine and iodine atoms.

*General Methods*

The present invention provides benzophenone-substituted triarylmethane -conjugated resins (including beaded resin supports), spacer-conjugated triarylmethane resins and libraries of peptides and polyamines linked to these spacer-conjugated triarylmethane resins. The invention also provides novel means of making and using these water-compatible solid supports and libraries. The skilled artisan will recognize that the methods of the invention can be practiced using a variety of ancillary and equivalent procedures and methodologies, which are well described in the scientific and patent literature., *e.g.*, Organic Syntheses Collective Volumes, Gilman *et al.* (Eds) John Wiley & Sons, Inc., NY; Venuti (1989) *Pharm Res.* 6:867-873. The invention can be practiced in conjunction with any method or protocol known in the art, which are well described in the scientific and patent literature. Therefore, only a few general techniques will be described prior to discussing specific methodologies and examples relative to the novel methods of the invention.

*Water-compatible solid support surfaces*

The compositions and methods of the invention incorporate the use of triarylmethane resins, including triphenylmethyl, or trityl, -based resins. The compositions and methods of the invention also incorporate the use of resins end-functionalized with a haloalkyl-terminated hydrophilic polyethylene glycol polymer. These resins can comprise polystyrene (PS), or other equivalent resins (see, e.g., U.S. Patent No. 5,290,819; 5,525,637; 5,591,778; 5,880,166; 5,900,146). For example, the polystyrene can comprise a poly(styrene-divinylbenzene) (PS-DVB) or an equivalent composition.

The hydrophilic polyethylene glycols can be branched or unbranched polymers. An example of an unbranched polyethylene glycol polymer is Tentagel™; various TentaGel resins are sold by, e.g., Rapp Polymere GmbH, Tübingen, Germany. Branched hydrophilic polyethylene glycol polymers include, e.g., Argogel™. Other examples include MOEPOP or a POEPOP.

*Screening biological molecules*

The invention provides methods of screening for and isolating polyamine binding molecules from samples, e.g., biological samples, using the resin-conjugated polyamide and polyamines combinatorial libraries of the invention. These methods can be used to isolate polyamine-binding molecules, e.g., peptides, polypeptides, carbohydrates (e.g., polysaccharides), lipids and nucleic acids (e.g., RNA and DNA). The libraries of the invention are particularly effective for screening for novel polyamine-binding molecules because they are constructed on a water-compatible support, they incorporate unnatural polyamines and, because a split-pool synthesis protocol was used in their synthesis, they are very diverse.

Polyamine ligands from the libraries of the invention that are identified to selectively bind to a biological molecules are useful as drugs. For example, some polyamines can be employed as gene delivery agents (see, e.g., Fujiwara (2000) Biochim. Biophys. Acta 1468(1-2):396-402) and as cytotoxic agents (see, e.g., Seiler (2000) Cell Biol. Toxicol. 16:117-130). Tumor cell growth id often accompanied by unusually high levels of polyamines; thus, polyamine-based inhibitors have been considered as anticancer agents (see, e.g., Manku (2000) J. Org. Chem. 66:874-885). Endogenous polyamines, such as spermine, have been found to cause block and modulation of a number of types of ion channels, such as inhibition of cation channels (see, e.g., Williams (1997) Biochem. J. 325:289-297. Polyamines act as neuroactive probes (see, e.g., Huang (2001) Biophys. J. 80:1262-1279). Polyamines were found to increase the translàtion of adenylate cyclase mRNA (see, e.g., Yoshida (2001) J. Biol. Chem. Jan 30; epub). Putrescine and spermine have been found to inhibit the proliferation of intraradical hyphae of fungi (see, e.g., Hrselova (2000) Folia Microbiol. (Praha) 45(2):167-71).

Examples of polyamines in nature include putrescine, spermidine, spermine and philanthotoxins (see, e.g., Stromgaard (2000) Chirality 12:93-102). Spermine has been reported to function directly as a free radical scavenger and has been associated with the neurotoxicity seen in Alzheimer's disease (AD) brains (see, e.g., Yatin (2001) J. Neurosci. Res. 63:395-401).

Cationic ammonium anions can establish ion pair interactions with the anionic phosphate groups on DNA and RNA, as well as with the side chain carboxylates of aspartic and glutamic acid residues. These ion pair interactions, however, are less geometrically directed than hydrogen bond interactions, thus at first sight are potentially less amenable to the design of selective ligands for biomolecules. On the other hand, multivalent interactions between a polyamine ligand and a receptor with multiple phosphates or carboxylates will impose a higher degree of geometrical restrictions and may help provide opportunities to isolate or develop highly selective polyamine ligands. The large polyamine libraries of the invention overcome current limitations in other combinatorial library systems (e.g, a mass spectrometry (MS) ladder sequencing which resolution limits the number of representative building blocks to around 20). These larger libraries are particularly useful in screening for RNA and polypeptide polyamine binding.

*Biological Molecules*

The invention provides methods for screening for, and isolating and characterizing, polyamine binding molecules in biological samples. The samples can be derived from any source. The sample can be derived from any tissue, cell or body fluid. Biological tissue samples can be pretreated, e.g., they can be tissue homogenates or extracts. Biological molecules that can be isolated by the methods of the invention include, e.g., lipids, nucleic acids (e.g., DNA, RNA), carbohydrates and polypeptides.

Biological samples can be crude tissue or cellular preparations or they can be purified biological molecules. The biological molecules can be tagged or labeled (e.g., fluorescent, radiolabeled, and the like) before application to the libraries of the invention.

## EXAMPLES

The following examples are offered to illustrate, but not to limit the claimed invention.

### Example 1: A Solid-Phase Method for the Synthesis of Chiral Polyamines

The following example describes an exemplary protocol for practicing the methods of the invention to prepare chiral polyamines.

*General procedures.* All Fmoc-amino acids and reagents employed are commercially available and were used without purification. All resins used were purchased from Rapp-Polymere (Tübingen, Germany) or NovaBiochem (San Diego, California). In most cases the loading value stated by the supplier was used. Solid-phase reactions that required heating were performed in glassware silanized by treatment with 20% TMSCl/toluene for >12 hours. Those done at room temperature (RT), including the iodine-promoted work-ups, were agitated inside polypropylene (PP) filter vessels. Tetrahydrofuran (THF) was dried by distillation over sodium/benzophenone ketyl. Anhydrous dimethylformamide (DMF) was obtained commercially and stored at $4^{O}C$ to reduce decomposition to dimethylamine and carbon dioxide. $^{1}H$ NMR spectra were recorded at 300 MHz in $CD_3OD$ while APT (Attached Proton Test) $^{13}C$ NMR spectra were recorded at 75.5 or 125 MHz in the same solvent (chemical shifts for both proton and carbon NMR are expressed in parts per million and were referenced against residual $CHD_2OD$). Signals arising from the trifluoroacetate counter-anions were not listed. $^{11}B$ NMR were acquired at 64.2 MHz in $CD_3OD$. Low resolution electrospray mass spectra were acquired using atmospheric pressure ionization (API) with a quadrupole detector (positive mode). High resolution (HRMS) analysis was obtained on a time-of-flight instrument. Specific conditions for HPLC analysis whether using UV or ES-MS detection were described for each compound concerned.

*General procedure for the synthesis of a polyamide on 1,3-diaminopropyl trityl resin.* The 1,3-diaminopropyl tritylpolystyrene resin was weighed into a polypropylene filter vessel and rinsed three times with DMF. A 0.5 M solution of 9-fluorenylmethoxy-carbonyl (Fmoc)-amino acid (2 equivalents with respect to the commercial loading of the resin) in DMF was then added to the resin. The suspension was vortexed for 2 to 5 minutes before the addition of a 0.5 M DMF solution of either 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and *N*-hydroxybenzotriazole ($HOBt{\cdot}H_2O$) (2 equivalents for coupling onto primary amines) or 2-(1H-7-azabenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) (2 equivalents for secondary amines). After an additional 2 to 5 minutes of vortexing, DIPEA (4 equivalents) was added. The

suspension was then agitated on an orbital shaker for 1 to 2 hours, drained and rinsed five times with DMF. The Fmoc protecting group was removed by treating the resin with 20 % piperidine in DMF two times – first for 5 minutes then for 30 minutes. The resin was then rinsed five times with DMF. Ninhydrin and bromophenol blue assay on the resin should be negative. Both the amino acid coupling and the Fmoc removal were repeated until the final amino acid was attached. To terminate the peptide sequence with an acetyl group the Fmoc group was first removed from the peptide. The resin was then swelled in DMF and $Et_3N$ (0.4 mL per gram of resin) was added. This suspension was vortexed for 2 to 5 minutes before the addition of acetic anhydride (1.0 mL per gram of resin). The suspension was agitated for 1 hour after which time the resin was drained and rinsed with DMF (3×), methanol (3×), and dichloromethane (5×). It was then dried under high vacuum for 16 hours.

*General procedure for the borane reduction/iodine work-up of resin-bound polyamides.* To the resin-bound polyamide, weighed inside a silanized round bottom flask, was added the 1.0 M $BH_3$/THF solution (minimum of 10 equivalents per amide) while under nitrogen atmosphere. The suspension was then refluxed gently (65°C) under nitrogen atmosphere until the reaction was complete (typically 24-72 hours). Upon cooling to room temperature the suspension was quickly transferred into a PP vessel via a silanized pipet using dry THF to rinse the flask. Dry THF, *i*-$Pr_2NEt$, and then glacial acetic acid were then added successively in a ratio of approximately 7:1:2 and for a total volume of ca. 10 mL/g resin. This was followed by the addition of iodine (3-5 equivalents per borane-amine adduct) as a concentrated THF solution. The vessel was then agitated on an orbital shaker for 2 to 4 hours (h) at room temperature. Afterwards the resin was filtered and rinsed with THF (3×), 1:3 $Et_3N$/DMF (3×), methanol (3×), and $CH_2Cl_2$ (5×) and dried under high vacuum for >12 hours to give the resin-bound neutral polyamine. The general procedure for the borane reduction/iodine work-up of resin-bound polyamides is illustrated in Figure 13.

*Typical cleavage of polyamide/polyamines from solid support (Method A: for compounds with unfunctionalized side chains).* To cleave the resin off the solid support, a portion was transferred into a round bottom flask and stirred in a 5% TFA/$CH_2Cl_2$ solution (10 to 20 mL per gram of resin) for 1 to 2 hours. The contents

were then filtered through glass wool (or a fritted glass funnel) and the resin rinsed thoroughly with 5% TFA/$CH_2Cl_2$ and methanol. The filtrate was evaporated and dried under high vacuum for >12 hours to afford the crude polyamine as a poly(trifluoroacetate) ammonium salt.

*Typical cleavage of polyamides/polyamines from solid support (Method B: for compounds functionalized or t-Bu/Boc protected side chains).* To a 0.5 g sample of the prepared resin bound, sulfur-containing polyamine in a round bottom flask, dry $CH_2Cl_2$ (5.0 mL) was charged (ca. 10 mL per gram of resin) followed by 50 μL of ethanedithiol (use 100 μL triisopropylsilane instead if the peptide or triamine does not contain sulfur) and 0.25 mL TFA. The resin was slurried for 0.5 h at RT and then filtered as above and rinsed into a round bottom flask with $CH_2Cl_2$ (3×) and 10 mL 1 : 1 $CH_2Cl_2$ : dry MeOH. The filtrate was concentrated under reduced pressure in a bath that is below 20°C. To the resulting residue, 20 mL of ether was slowly added while vigorously swirling the flask. The ether was then decanted and the remaining residue was placed under high vacuum for 12 h to provide the polyamine product as a poly(trifluoroacetate) ammonium salt.

*Optimization of a new oxidative work-up for the cleavage of resin-bound borane-amine adducts.* Preliminary investigations on the iodine-promoted work-up were carried out using trityl-polystyrene bound $H_2N(CH_2)_3NH$-LPhe-LAla-Ac tripeptide **1**, Figure 2); aliquots of all resin-bound peptide precursors were cleaved from the resin and provided satisfactory analytical data (ES-MS, $^1H$ NMR) (see below). Resin-bound peptide precursors were easily assembled from 1,3-diaminopropyl tritylpolystyrene using standard methods for peptide synthesis with Fmoc-amino acids. For the reduction step, the use of a large excess of concentrated $BH_3$ (> 40 equiv.) was found necessary to ensure complete reduction in a relatively short time. A reaction time of 24 hours at 65 °C under nitrogen atmosphere was found sufficient for the reaction of model tripeptide substrate **1, Figure 2,** as evidenced by the absence of amide absorption by single bead Fourier transform infrared (FT-IR) microscopy, and by the absence of $[MH+n14]^+$ signals from underreduced residues in the electrospray mass spectrum (ES-MS) of cleaved tetraamine **4, Figure 2**. Longer reduction times, however, maybe necessary for longer polyamides and sterically

hindered residues like valine (*vide infra*). Efforts to increase the rate of polyamide reduction with the use of borane-DMS in refluxing toluene (see, e.g., Brown (1981) Synthesis:996-997) were unsuccessful. In addition, the combined use of equimolar amounts of borane, methyl-borate and boric acid as described by Nefzi (1997) Tetrahedron Lett. 38:931, failed to give any reduction.

Prior to the work-up step, excess borane is rinsed away with dry THF. The resin is then suspended in the 7:2:1 THF/AcOH/DIPEA buffer system used for the actual work-up. The use of buffered media, i.e., the presence of a trialkylamine, is required in order to trap the iodohydric acid released in the process, whereas the use of excess acetic acid is required both as a protic/nucleophilic source and for maintaining a relatively mild pH (see mechanistic studies below). The aminotrityl linkage is fully tolerant of these conditions. A major proportion of THF is employed in order to allow sufficient swelling of the resin. Immersion of the resin is immediately followed by addition of excess iodine; typically 3 to 5 equiv. per amide, added in a concentrated THF solution (in some cases where lower amounts of iodine (e.g. 2 equiv./borane-amine adduct) was used, a minor ($M^+$+24) peak corresponding to the molecular ion plus $B_2H_2$ was observed in the resulting ES-MS spectra of cleaved polyamine products. The use of larger amounts of iodine tends to suppress that peak). After a few hours, the resin is rinsed successively with THF, with a basic neutralizing solution (3:1 DMF/$Et_3N$) to form the free resin-bound tetraamine **2, Figure 2,** then with methanol and dichloromethane. Whereas a bromophenol blue test performed prior to the oxidative work-up is negative, the same test shows a positive outcome when performed at this final stage, thus hinting at the successful generation of a free polyamine. Supported product **2, Figure 2** was cleaved from the resin with dilute trifluoroacetic acid to give tetraamine **3, Figure 2** as a poly(trifluoroacetate) ammonium salt. Alternatively, it was fully acetylated to afford compound **4, Figure 2** after cleavage. Both reduced peptide derivatives **3** and **4 of Figure 2** were obtained as crude material in high overall yield and were fully characterized. Analysis by RP-HPLC under both UV and ES-MS detection confirmed their high degree of purity (91% and 95% respectively). At this stage, a comparison of the iodine-promoted work-up with a borane-exchange protocol was carried out. To this end, model tripeptide **1, Figure 2**

was reduced, then treated using either: a) the above conditions with iodine, b) neat piperidine for 24 hours at 65 °C (for examples of peptide reduction/ piperidine exchange on methylbenzhydrylamine polystyrene resin followed by HF cleavage, see, e.g., Nefzi (2000) Tetrahedron 56:3319-3326; Nefzi (2000) Tetrahedron Lett. 41:5441-5446; Nefzi (1999) Tetrahedron 55:335-344), and c) direct addition of acetic anhydride to the resulting oligo(borane-amine) adducts ($Ac_2O$, $Et_3N$). Interestingly, acetic anhydride appeared to act as an electrophilic reagent of sufficient strength to breakdown the borane-amine adducts and subsequently acylate the resulting secondary amines to provide **4, Figure 2** in 78% crude yield, although with a lower purity (84%). The resin-bound products from the iodine-promoted work-up and the piperidine-exchange were independently acetylated to afford **4, Figure 2** in quantitative yields. Both methods were found comparable as isolated crude materials from the borane-exchange work-up provided purity of 89% and 94% for products **3** and **4** of **Figure 2** respectively.

Although previous reports hint at the absence of epimerization in borane promoted reductions of peptide derivatives (see, e.g., Roeske (1976) J. Org. Chem. 41:1260; Northrop (1977) J. Org. Chem. 42:4148; Chu (1991) J. Org. Chem. 56:5196; Cuervo, et al., In Peptides, 1994, Proceedings of the 23rd European Peptide Symposium, Maia, H.L.S. Ed.; ESCOM, Leiden, 1995, p. 465), experiments were performed to ascertain this by synthesizing all four diastereomers of supported tripeptide 1, **Figure 3** (aliquots of all resin-bound peptide precursors were cleaved from the resin and provided satisfactory analytical data (ES-MS, $^1$H NMR) (see below). After reduction/oxidative work-up, the resulting diastereomeric tetraamines (**Figure 3**) were compared by NMR, optical rotation, and analyzed by RP-HPLC to quantify the extent of epimerization at the side-chain centers. For instance, were "LL-**3**" to show partial epimerization at any one or both of its two stereogenic centers, the availability of all resulting diastereomers would allow detection and quantitation of racemization. Should it undergo complete racemization at all centers, two peaks of equal intensity corresponding to two mixtures of enantiomeric tetramines would be detected. The diastereomers were analyzed by high pressure liquid chromatography (HPLC) analyses; optimal conditions for HPLC analysis were found where sets of tetraamine

diastereomers are clearly separated. As shown in **Figure 4**, **LD-3** and **DD-3** gave effective separation when co-injected as an equimolar mixture. Yet, both give single peaks when injected independently. The **DL-3**/**LL-3** pair gave an identical outcome, indicating the absence of any appreciable racemization in this borane reduction/oxidative work-up process. It remains unknown, however, whether all chiral amino acid residues behave similarly.

*Scope of compatible linkers and supports.* Houghten and co-workers, as described in see, e.g., Nefzi (2000) Tetrahedron 56:3319-3326; Nefzi (2000) Tetrahedron Lett. 41:5441-5446; Nefzi (1999) Tetrahedron 55:335-344, have described peptide reductions on a methylbenzhydryl support that can withstand the strongly aqueous acid conditions employed to breakdown the resulting borane-amine adducts. Unfortunately, this particular linker requires HF cleavage techniques to liberate the polyamine product; to be performed safely, these procedures must use special apparatus.

In contrast, the methods of the invention do not use or require harsh conditions for working on trityl-based linkers. The loading of amines and other functionalities onto chlorotrityl resins is straightforward, and eventual cleavage of products can be carried out conveniently using dilute TFA, as described by, e.g., Nash (1966) Tetrahedron Lett. 37:2625-2628.

The methods of the invention also use a Rink resin linker, as shown in Figure 5. Supported tetraamide Figure 5, compound **5** (aliquots of all resin-bound peptide precursors were cleaved from the resin and provided satisfactory analytical data, ES-MS, $^1$H NMR) was reduced and treated as described above, but provided tetraamine **Figure 5**, compound **3** after cleavage of the resin, with a noticeably lower purity and yield as compared to the trityl resin example described above. In addition, ester-based linkers, such as a hydroxymethylphenoxy handle, were found unsuitable, as the use of a large excess of borane under extreme conditions of temperature and time leads to significant levels of reductive cleavage. As expected, these stringent reaction conditions limit the possible choices of linker and support.

For applications for screening water-soluble biomolecules against large libraries of bead-supported polyamines, the suitability of polystyrene-polyethylene

glycol copolymers (e.g Tentagel) was examined. Very few commercial PS-PEG resins are available with a trityl linker; most or all of these resins have the trityl linker attached either via a 4-carboxamide or a 4-phenoxy anchor. All attempts with the latter type met with failure as none or little polyamine product was recovered following reduction, work-up and cleavage of the resin. The commercial hydrophilic trityl-derivatized supports were tried, but did not work well because they are linked through a phenoxymethylene or a carboxamide that did not tolerate the borane. The carboxamide worked sometimes but it was found to be irreproducible. This is thought to be due to the lack of chemical compatibility between borane under the conditions employed, and many other functionalities. Therefore, the present borane-proof linker with only saturated non-functionalized groups to attach the trityl unit to the hydrophylic support was developed. The use of saturated methylene (CH2) groups has been found to be best. An explanation to the chemoselectivity problems with the commercial trityl resins is that the phenoxymethylene group is reducible under these conditions, leading to premature release of the resin-bound material. On the other hand, as demonstrated with the synthesis of tetraamine compound **3, Figure 5** from supported tripeptide compound **1', Figure 5,** the 4-carboxamide derivatized PS-PEG-trityl resin was found resistant to the reduction conditions, although lower yield (65%) and purity (62%) were obtained as compared to the use of trityl-PS (Figure 5). Moreover, it is likely that the linker's 4-carboxamide group also gets reduced to the corresponding secondary amine. This may be undesirable for on-bead screening applications and cases where the supported polyamine product is needs to be further functionalized selectively.

*Side-chain generality.* The chemoselectivity of diborane has been well documented in the literature (see, e.g., Brown (1982) J. Org. Chem. 47:3153-3163; Brown (1973) J. Org. Chem. 38:912). However, in order to assess the scope of compatible side-chain functionalities from α-amino acid components, the combined effect of borane with the iodine-promoted work-up was examined. To this end, a series of model dipeptides **6** were synthesized and treated as described above (Figure 6) (aliquots of all resin-bound peptide precursors were cleaved from the resin and

provided satisfactory analytical data, ES-MS, $^{1}H$ NMR). Table 1 sets forth exemplary results of this side chain generality study.

**Table 1. Side-chain generality study.**[a]

| entry | peptide substrate | triamine product | yield[b] (%) | purity[c] (%) | ES-MS analysis calcd | obsd |
|---|---|---|---|---|---|---|
| 1 | 6a (Val) | 8a | 79 | >90 | 188.2 | 188.2 |
| 2 | 6b (Phe) | 8b | 75 | 95 | 236.2 | 236.2 |
| 3 | 6c (*t*-Bu-Tyr) | 8c[d] | 70 | >90 | 252.2[d] | 252.2 |
| 4 | 6d (*t*-Bu-Ser) | 8d | 61 | 90[e] | 232.2 | 232.2[e] |
| 5 | 6e (*t*-Bu-Cys) | 8e | 79 | >95 | 248.2 | 248.2 |
| 6 | 6f (Met) | 8f | 90 | 90 | 220.2 | 220.2 |
| 7 | 6g (*t*-Bu-Asp) | 8g[f] | 81 | -[f] | 222.2 | -[f] |
| 8 | 6h (*t*-Bu-Glu) | 8h[f] | 79 | -[f] | 236.2 | -[f] |
| 9 | 6i (Gln) | 8i[g] | 95[h] | >80[h] | 203.2[g] | 203.2 |
| 10 | 6j (Boc-Trp) | 8j | 79 | 95 | 375.3 | 375.3 |
| 11 | 6k (Boc-His) | 8k | - | - | 326.3 | - |

[a] General reaction conditions: typical scale 0.4 g resin **6**, 1M $BH_3$, 65 °C, 1-5 d; work-up: 2-3 equiv. $I_2$ in THF-AcOH-DIPEA 5:2:1. [b] Non-optimized yields after cleavage from the resin (5% TFA/$CH_2Cl_2$, additives used when necessary) and one round of precipitation with ether. [c] Purity was estimated by $^{13}C$ NMR (peak height comparison between peaks from products **8** and unknown ones). [d] Significant loss of the *t*-Bu group was observed in the resin cleavage operation so it was characterized as a free phenol. [e] A 5-10% loss of the *t*-Bu group was observed. [f] The ester side chain undergoes modifications whereby a mixture of the corresponding *t*-butyl ether and primary alcohol is obtained. [g] The glutamine side chain undergoes a modification to the corresponding primary amine. [h] Crude yield and purity (no ether precipitation).

In this side chain generality study, as summarized in Table 1, the tyrosine-based dipeptide **6c** showed no sign of electrophilic ortho-iodination (entry 3). Similarly, tert-butyl protected cysteine and methionine residues of **6e** and **6f** were found to be resistant under the oxidative work-up, and afforded respectively triamines **8e** and **8f** in excellent yields and purity (entries 5 and 6). Ester-containing dipeptides **6g** and **6h** respectively made of protected Asp and Glu residues gave products **8g** and **8h** as mixtures containing variable proportions of the free alcohol side chain (from

ester reduction) and the corresponding *t*-butyl ether (entries 7,8). None of the polyamine with intact *t*-butyl ester group was observed. Indeed, it is known from the literature that the reduction of esters with proximal basic groups can lead to anomalous reduction products such as ethers (see, e.g., Kornet (1968) J. Org. Chem. 33:3637). As expected, the primary amide of glutamine-containing dipeptide **6i** was reduced to triamine **8i** with the corresponding side chain of ornithine (entry 9). Whereas tryptophan-containing substrate **6j** resisted our conditions and provided triamine **8j** cleanly (entry 10), histidine (i.e. **6k**) underwent degradation whether protected or not (entry 11). It is not clear at this point whether these problems originate from the reduction step or the iodine-promoted work-up. Valine-containing polyamides such as dipeptide **6a** tend to undergo reduction at a slower rate (entry 1). For such hindered residues we have found it preferable to use longer reaction times (e.g., 72+ hours). Although crude materials were found of reasonable purity, all triamine tris(trifluoroacetatic acid) salt products described in Table 1 were further purified by ether precipitation and fully characterized. They were obtained in moderate to high yields and high purity by $^{13}C$ NMR analysis, although slight loss of the *t*-butyl-based side chain protective groups is occasionally observed as a consequence of the resin cleavage operation using 5% TFA/$CH_2Cl_2$ (the use of methanol has been found effective to rinse off products from the resin and provide increased yields; however, in the evaporation of filtrates containing dilute TFA in dichloromethane, methanol also tends to give increased cleavage of the t-Bu and Boc protecting groups). In the case of tyrosine-containing triamine **8c**, significant loss of the *t*-butyl group was found unavoidable thus it was characterized as its unprotected form.

*Reduction of tertiary amides: an approach to oligo(tert-amines).* In principle, access to oligomeric tertiary amines could arise from the exhaustive reduction of a polypeptide made of tertiary amide residues. However, as a result of sluggish coupling rates between terminal secondary amines and activated aminoacids, tertiary amides are difficult to synthesize using standard amide coupling methods. Therefore, the use of oligo(*sec*-amines) as precursors for oligo(*tert*-amines) was examined. Indeed, oligo(*sec*-amines) could be acylated to provide tertiary polyamide derivatives, and consequent reduction would provide the oligo(*tert*-amines) with a

constant lateral chain. This two-step approach to oligo(*tert*-amines) from model triamide **9** (Figure 7) was tested. The latter was obtained from acylation of **2** with propionyl chloride (aliquots of all resin-bound peptide precursors were cleaved from the resin and provided satisfactory analytical data, ES-MS, $^{1}H$ NMR). The borane reduction of **9**, however, turned out to be very sluggish, giving a complex mixture of partial reduction products with little of the desired tetraamine **11** after 5 days at 65°C. The reduction of a polybenzoylated analogue gave a similar outcome.

The direct reaction of oligo(borane-amine) intermediate **12** (Figure 8) with excess propionaldehyde was attempted (borane-amine adducts can serve as hydride donors for the reductive amination of aldehydes, see, e.g., Lane (1973) Aldrichimica Acta 6:51; Hutchins (1984) Org. Prep. Proc. Int. 16:335; Carboni (1999) Tetrahedron 55:1197-1248) (intermediate **12** is the product from the reduction of **1** not subjected to any work-up). Although some fully alkylated product **11** was obtained after cleavage of the resin, there were significant amounts of incomplete alkylation. Ultimately, this was solved by the in situ addition of sodium triacetoxyborohydride as external hydride source, providing tetraamine **11** in high yield and purity. The use of a branched aldehyde, isobutyraldehyde, was also successful and afforded **14** cleanly.

The same procedure performed with benzaldehyde resulted in large proportions of the putative, bridged *N,N*-acetals under all conditions examined. Apparently, this intramolecular side-reaction competes with hydride addition to the iminium intermediate in the case of aromatic aldehydes.

The reduction of model tripeptide **15** with a central tertiary amide was also investigated and resulted in a rather unexpected outcome (Figure 9,); aliquots of all resin-bound peptide precursors were cleaved from the resin and provided satisfactory analytical data (ES-MS, $^{1}H$ NMR). According to $^{1}H$ and $^{13}C$ NMR as well as ES-MS, the usual iodine-promoted work-up and resin cleavage steps led to the isolation of triaminoborane **19** as major product. The $^{11}B$ NMR spectra confirmed the presence of boron with a chemical shift (18.3 ppm) consistent with a tetracoordinated species. An extra work-up operation was required in order to extrude boron by transesterification with ethylene glycol and isolate the desired tetraamine **20**. The anomalous behavior of triamide **15** may be due to the faster reduction rate of tertiary

amides with diborane. It may be that the borane-amine adduct **16** is formed initially, allowing a facile double intramolecular hydride delivery to give intermediate **17**. The latter may also absorb excess borane to give **18** as end-product prior to the work-up.

*Mechanistic studies and comparison of various work-up protocols.* The invention's novel oxidative work-up for borane-amine cleavage was analyzed mechanistically. First, evidence was generated to explain the efficiency and exact role of the buffered iodine solution. In fact, the trifluoroacetic acid used to release the product from the support alone could cleave the multiple borane-amine adducts resulting from the solid-phase reduction of polyamides. The question of whether the buffered iodine was effective, or whether it was the TFA used in the product release operation that cleaves the borane-amine adducts was explored. This issue is relevant toward applications requiring bead-supported free polyamines for screening purposes. By avoiding the TFA cleavage operation necessary in solid-phase reductions, the solution-phase reduction of amino esters unarguably addressed this first issue. For instance, in **Figure 10**, *N*-acetyl amino acid ester **21**treated under the usual reduction conditions followed by the buffered iodine work-up obtained a high yield of crude *N*-alkylated product **22** with no traces of borane-amine adduct (Figure 10(A)). U.S. Patent 3,338,726, described another protocol using iodine to cleave borane-amine adducts; specifically, a method for the quantitative determination of borane-amines using iodine in a sodium acetate/acetic acid aqueous buffer was described. In this method, the borane is titrated with 3 equivalents of iodine in the presence of starch, giving a sharp and rapid end point; boric acid, the amine, and six iodide ions are produced (Figure 10(B)).

As indicated above, the work-up procedure of the invention is used in buffered organic solution to allow resin swelling. The use of excess iodine was favored to ensure completion of borane-amine cleavage in a minimal time. It had been previously observed that amounts of iodine beyond one equivalent led to decolorization of the solution from purple to yellow and even clear. This may occur because the three hydrids from the borane-amine adducts are all potentially reducible. However, it was further investigated how many equivalents of iodine are truly necessary to ensure total cleavage of the borane-amine adducts. To this end, a simple model secondary amide

(**23**) was synthesized for solution-phase studies (Figure 11). For comparison purposes, a sample of the amine product **26** was synthesized following a strong acid work-up and basic extractions (Table 2, entry 1). In addition, the corresponding borane-amine adduct **25** was made independently from the reaction of amine **26** with one equivalent of borane. A series of test reductions of **23** with a slight excess of borane (2.2 equiv.) in THF was performed, followed by a work-up with a variable amount of iodine in the usual buffered solution (excess 2:1 AcOH/DIPEA buffer added to the THF reaction mixture). Unless indicated otherwise, the reaction products were isolated following addition of aqueous base containing some thiosulfate (to neutralize any leftover iodine), and multiple extractions with diethyl ether. First, a control experiment in the absence of buffered iodine solution revealed that borane-amine adducts were cleaved to the extent of about 20 % under the basic extraction conditions used to recover crude materials (entry 2). Also, a control work-up with 1:2:5 DIPEA/AcOH/THF for two hours in absence of iodine revealed substantial cleavage (40%) of the borane-amine adduct to give amine **26** (entry 3). Thus, by difference with the value obtained from entry 2, it appears that the acetic acid-acetate buffer alone can be accounted for approximately 20% cleavage of the borane-amine adduct **25**. The work-up trial with 0.5 equiv. iodine gave 70% cleavage (entry 4). Finally, **25** was entirely transformed to **26** using only one equivalent of iodine (entry 5). Background cleavage makes it difficult to quantify the exact amount of borane-amine cleaved through the single action of iodine. However, these results confirm that iodine is required for achieving full cleavage within a reasonable timeframe, and that a single equivalent can be sufficient to obtain the desired effect. The use of excess iodine, however, is advisable in order to accelerate solid-supported reactions.

The above observations support a mechanistic pathway for borane-amine cleavage by iodine, as illustrated in Figure 12 (however, the invention is not limited by any particular pathway or mechanism of action). First, the presence of any oxygen-based nucleophile such as acetate ion from the buffer is very likely to cleave the aminoborane unit of adduct **I** rapidly. Aminoborane compounds are known to be very labile whenever opportunity to form a new, thermodynamically favored B-O bond is present; see, e.g., Steinberg, H.; Brotherton, R.J. Organoboron Chemistry, Vol. 2;

John Wiley and Sons: New York, NY, 1966. In fact, the borane-amine adduct isolated after reduction of **23** without oxidative workup (entry 2) is identical to the authentic sample of **25** made directly by adding borane to amine **26**. This indicates that the aminoborane bond of **24** is hydrolyzed in the basic aqueous extractions performed to isolate crude materials.

From the resulting borane-amine adduct **II**, increasing equivalents of iodine may be expected to substitute each hydride successively to give the monoiodoborane (**III**), diodoborane, and ultimately the triiodoborane-amine adduct (the direct synthesis of monoiodo- and diiodoborane-amine adducts from the corresponding borane-amines and the required amount of iodine has been described by Nöth (1960) Chem. Ber. 93:2251-2263; Nöth (1964) Chem. Ber. 97:110-118). There are very few relative kinetic measurements available for the acidic hydrolysis of these compounds (see, e.g., Ryschkewitsch (1960) J. Am. Chem. Soc. 82:3290-3294; Nöth, H.; in Progress in Boron Chemistry, Vol. 3, Eds. Brotherton, R.J. and Steinberg, H.; Pergamon Press:Oxford, 1970, Chapter 4, pp. 211-312). However, the accepted order of Lewis acidity for trihaloboranes is $BF_3 < BH_3 < BCl_3 < BBr_3 < BI_3$. (see, e.g., Noth (1970) supra; Young (1966) J. Am. Chem. Soc. 88:4390-4396). Consequently, substitution of hydrides in **II** for iodides is expected to strengthen the amine adducts and reduce their susceptibility to dissociate and undergo protolysis by the acid from the buffer. On the other hand, solvolysis by amines (see Douglass (1964) J. Am. Chem. Soc. 86:5431; Ryschkewitsch (1967) J. Am. Chem. Soc. 89:3145), and substitution of iodine in monoiodoborane amine adducts by charged nucleophiles (see, e.g., Bratt (1974) J. Chem. Soc, Dalton Trans. 2161-2163; Mills (1989) J. Chem. Soc., Chem. Commun. 900-901) have been shown possible. Vedrenne (1999) J. Am. Chem. Soc. 121:1090-1091, proposed that the mechanism is identical to an $SN_2$ reaction on a primary alkyl halide. However, because the results described above demonstrated that a single equivalent of iodine is necessary for cleavage, it is hypothesized that the monoiodide **III** is displaced by the acetate anion (present in large excess from the buffer) to give the acetoxyborane adduct **IV** (bold arrows in Scheme 9). However, due to the backbonding of oxygen, the latter is a much weaker adduct than the monoiodoborane adduct, and is thus expected to dissociate readily. Under such

circumstances, acetic acid would be strong enough a protic source to trap the free amine irreversibly, and lead to solvolysis of the vulnerable, uncomplexed acetoxyborane. The use of methanol in place of acetic acid has also been tested, but under all conditions tried, with or without iodine, no cleavage was observed over background (entries 6 to 8, **Table 2**), although decolorization of the solution lends to the suggestion that iodoborane-amine species are formed here as well. This points out for the need of a protic/nucleophilic source of sufficient strength in our work-up procedure.

Other work-up conditions reported in the literature were also evaluated. The use of trifluoroacetic acid (Choi (1991) Tetrahedron Lett. 32:5517-5520), albeit not applicable to the synthesis of trityl resin-bound polyamines, is effective. On the other hand, basic aqueous conditions for borane exchange were found ineffective at room temperature. Similarly, the use of a large excess of a tertiary amine like diisopropylethylamine to effect cleavage of **25** by borane exchange was unsuccessful under conditions comparable to the oxidative work-up (RT, 2 hours). Various attempts using phosphines and phosphites were also futile. On the other hand, similar treatment with the secondary amine morpholine resulted in complete cleavage to **26**. It remains uncertain if these particular conditions are equally efficient and practical on solid-phase at room temperature. However, this is probably not the case as Houghten and co-workers (in Nefzi (2000) Tetrahedron 56:3319-3326; Nefzi (2000) Tetrahedron Lett. 41:5441-5446; Nefzi (1999) Tetrahedron 55:335-344) described the use of piperidine for their work-up of solid-phase reductions at 65 °C for >12 hours.

*12-Amino-(4S)-methyl-(7S)-benzyl-3,6,9-triazaundecane tetrakis (trifluoro-acetatic acid) salt (LL-3).* Resin-bound triamide **1, Figure 1** (0.351 g, 0.259 mmol, 0.74 mmol/g) was reduced as described in the general procedure to give the supported tetraamine **2**. A portion of the tetraamine (0.174 g, 0.134 mmol, 0.77 mmol/g) was cleaved to provide 95 mg (crude yield of 95 %) of **LL-3** as a yellow oil. Purity by HPLC was 82 % (a/a) (depending on reaction scale and HPLC conditions a typical range of purity obtained for crude **3** was 80-95%). HPLC conditions; column: Zorbax XDB-C8 (4.6 × 50 mm, 3.5 μm); eluent: 10% acetonitrile (0.1% TFA) and 90% water (0.1% TFA) to 20% acetonitrile over 15 minutes at 0.70 mL/min; column

temperature: 20°C; detection: UV diode array at 250 nm; $R_t$ = 8.109 min. $^1$H NMR (300 MHz, $CD_3OD$) δ: 7.38 - 7.18 (m, 5H), 3.24 - 2.84 (m, 12H), 2.79 (dd, $J$ = 4.2, 13.5 Hz, 1H), 2.63 (dd, $J$ = 7.8, 13.5 Hz, 1H), 2.20 - 2.00 (m, 2H), 1.31 (t, $J$ = 7.3 Hz, 3H), 1.27 (d, $J$ = 6.8 Hz, 3H). APT $^{13}$C NMR (75.5 MHz, $CD_3OD$) δ: 138.5 (C), 130.3 (CH), 129.9 (CH), 127.9 (CH), 58.2 (CH), 54.9 (CH), 52.2 ($CH_2$), 49.2 ($CH_2$), 45.9 ($CH_2$), 41.3 ($CH_2$), 39.5 ($CH_2$), 37.8 ($CH_2$), 25.2 ($CH_2$), 14.8 ($CH_3$), 11.5 ($CH_3$). ESMS *m/z* 315.5 ($M^+$+Na), 293.5 (M+H), 147.6 (($M^+$+2H)/2). HRMS (ES) for $C_{17}H_{33}N_4$ ($M^+$+H) calcd 293.270522, found 293.271472. IR (methanol cast) 3001 (N-H stretches), 1675, 1202, 1131, 799, 721. $[\alpha]_D$ = (+) 14.6° (c = 76.8 mg/mL in MeOH).

*12-Amino-(4R)-methyl-(7R)-benzyl-3,6,9-triazaundecane tetrakis(trifluoroacetatic acid) salt, (DD-3).* Crude yield: 92.5%. Purity by HPLC (same conditions as for **LL-3**): 86% (a/a); $R_t$ = 8.234 min. $^1$H NMR (300 MHz, $CD_3OD$) δ: 7.40 - 7.20 (m, 5H), 3.22 - 2.84 (m, 12H), 2.80 (dd, $J$ = 4.2, 13.5 Hz, 1H), 2.63 (dd, $J$ = 7.8, 13.5 Hz, 1H), 2.18 – 2.00 (m, 2H), 1.31 (t, $J$ = 7.2 Hz, 3H), 1.28 (d, $J$ = 6.6 Hz, 3H). $^{13}$C NMR (75.5 MHz, $CD_3OD$) δ: 138.5, 130.3, 130.0, 128.0, 58.2, 54.9, 52.0, 49.2, 45.9, 41.3, 39.3, 37.8, 25.2, 14.8, 11.5. ESMS *m/z* 315.5 ($M^+$+Na), 293.5 ($M^+$+H). HRMS (ES) for $C_{17}H_{33}N_4$ ($M^+$+H) calcd 293.270522, found 293.270779. IR (methanol cast) 3006 (N-H stretches), 1673 cm$^{-1}$. $[\alpha]_D$ = (-) 14.7° (c = 24.3 mg/mL in MeOH).

*12-Amino-(4S)-methyl-(7R)-benzyl-3,6,9-triazaundecane tetrakis(trifluoroacetatic acid) salt (LD-3).* Crude yield: 99.5%. Purity by HPLC (same conditions as for **LL-3**): 78% (a/a); $R_t$ = 9.261 min. $^1$H NMR (300 MHz, $CD_3OD$) δ: 7.40 - 7.20 (m, 5H), 3.24 - 2.82 (broad m, 12H), 2.65 (dd, $J$ = 7.2, 7.2 Hz, 1H), 2.61 (dd, $J$ = 7.8, 7.8 Hz, 1H), 2.14 – 2.00 (m, 2H), 1.30 (t, $J$ = 7.5 Hz, 3H), 1.29 (d, $J$ = 7.2 Hz, 3H). $^{13}$C NMR (75.5 MHz, $CD_3OD$) δ: 138.5, 130.7, 130.0, 128.0, 58.4, 54.9, 52.0, 50.7, 45.9, 40.6, 39.2, 37.8, 25.2, 14.5, 11.6. ES-MS *m/z* 315.5 ($M^+$+Na), 293.5 ($M^+$+H). IR (methanol cast) 3006 (N-H stretches), 1675 cm$^{-1}$. HRMS (ES) for $C_{17}H_{33}N_4$ ($M^+$+H) calcd 293.270522, found 293.270182. $[\alpha]_D$ = (+) 1.45° (c = 25.2 mg/mL in MeOH).

*12-Amino-(4R)-methyl-(7S)-benzyl-3,6,9-triazaundecane tetrakis(trifluoroacetatic acid) salt (DL-3).* Crude yield: 95%. Purity by HPLC (same conditions as for **LL-3**): 80% (a/a); $R_t$ = 9.012 min. $^1$H NMR (300 MHz, $CD_3OD$) δ: 7.40 - 7.20 (m, 5H), 3.24 - 2.82 (broad m, 12H), 2.65 (dd, $J$ = 8.4, 9.9 Hz, 1H), 2.60 (dd, $J$ = 8.4, 8.4 Hz, 1H), 2.14 – 2.00 (m, 2H), 1.30 (t, $J$ = 7.5 Hz, 3H), 1.29 (d, $J$ = 6.6 Hz, 3H). $^{13}$C NMR (75.5 MHz, $CD_3OD$) δ: 138.5, 130.3, 130.0, 128.0, 58.4, 55.0, 52.0, 50.7, 46.0, 40.6, 39.3, 37.8, 25.2, 14.5, 11.8. ESMS *m/z* 315.5 ($M^+$+Na), 293.5 ($M^+$+H). HRMS (ES) for $C_{17}H_{33}N_4$ ($M^+$+H) calcd 293.270522, found 293.270825. IR (methanol cast) 3006 (N-H stretches), 1674 $cm^{-1}$. $[\alpha]_D$ = (-) 1.17° (c = 28.3 mg/mL in MeOH).

*$N^{3,6,9}$-Triacetyl-12-amino-(4S)-methyl-(7S)-benzyl-3,6,9-triazaundecane trifluoroacetatic acid salt (4).* The supported tetraamine **2** (85.8 mg, 0.072 mmol, 0.84 mmol/g) was swelled in 1.0 mL of DMF. $Et_3N$ (0.21 mL, 1.51 mmol) was then added and the resin vortexed for one minute before the addition acetic anhydride (0.34 mL, 3.60 mmol). The suspension is agitated for 16 hours and then rinsed with DMF (3×), methanol (3×), dichloromethane (5×), then dried under high vacuum for >12 hours to give the resin-bound triacetamide (0.130 g). A portion of the resin (26.4 mg, 0.020 mmol, 0.76 mmol/g) was cleaved from the support with 5 % TFA/$CH_2Cl_2$ as described above to afford 10.6 mg (100% crude yield) of **4**. Purity by HPLC: 95% (a/a). HPLC conditions; column: Zorbax SB-C8 (4.6 × 50 mm, 3.5 μm); eluent: 10% acetonitrile (0.1% TFA) and 90% water (0.1% TFA) to 40% acetonitrile over 4 minutes and maintained for an additional 6 minutes at 0.90 mL/min; column temperature: 20°C; detection: UV diode array at 210 nm; $R_t$ = 5.649 min. $^1$H NMR (300 MHz, $CD_3OD$) δ (complex mixture of 8 amide rotomers). IR (methanol cast) 2984.34 (N-H stretches), 1677, 1617. HRMS (ES) for $C_{23}H_{39}N_4O_3$ ($M^+$+H) calcd 419.302217, found 419.301333.

*(4R)-Isopropyl-9-amino-3,6-diazanonane tris(trifluoroacetatic acid) salt (8a).* 1,3-Diaminopropane linked *N*-acetyl-L-valine trityl resin **7a** was constructed from **6a** as described above. Cleavage of the triamine product off of the resin (0.38 g, 0.37 mmol) was accomplished using method A mentioned above. After ether precipitation, an oily white solid corresponding to **8a** was obtained (0.16 g, 79% crude

yield). $^1$H NMR ($CD_3OD$) δ: 3.47 (m, 1 H), 3.41 (d, $J$ = 3.5 Hz, 2H), 3.31 – 3.17 (m, 4H), 3.07 (t, $J$ = 7.9, 7.5 Hz, 2H), 2.28 (m, 1H), 2.14 (m, 2H), 1.36 (t, $J$ = 7.2 Hz, 3H), 1.10 (d, $J$ = 6.6 Hz, 3H), 1.05 (d, $J$ = 7.0 Hz, 3H); $^{13}$C NMR ($CD_3OD$) δ: 61.7, 47.0, 46.9, 43.1, 37.8, 28.7, 25.5, 18.3, 16.6, 11.4; HRMS (ES) for $C_{10}H_{26}N_3$ ($M^+$+H) calcd 188.212673 found 188.212895. $[\alpha]^{25}{}_D$ = (+) 10.0° (c = 2.41, MeOH).

*(4S)-Benzyl-9-amino-3,6-diazanonane tris(trifluoroacetatic acid) salt (8b).* Phenylalanine-derivatized resin **7b** was constructed from **6b** as described above. Cleavage of the triamine product off of the resin (0.44 g, 0.41 mmol) was accomplished using method A. After ether precipitation, an off-white solid corresponding to **8b** was obtained (0.18 g, 75% crude yield). $^1$H NMR ($CD_3OD$) δ: 7.5 (m, 5H), 3.93 (m, 1H), 3.51 (dd, $J$ = 14.2, 7.7 Hz, 1H), 3.30 – 2.95 (m, 5H), 3.11 (t, $J$ = 7.6 Hz, 2H), 3.03 (t, $J$ = 7.7 Hz, 2H), 2.08 (m, 2H), 1.32 (t, $J$ = 7.2 Hz, 3H). $^{13}$C NMR ($CD_3OD$) δ: 135.9, 130.4, 130.3, 128.9, 57.9, 48.9, 46.8, 42.3, 37.7, 36.0, 25.4, 11.6; HRMS (ES) for $C_{14}H_{26}N_3$ ($M^+$+H) calcd 236.212673 found 236.212665. $[\alpha]^{25}{}_D$ = (+) 8.52° (c = 6.19, MeOH).

*(4S)-p-Hydroxybenzyl-9-amino-3,6-diazanonane tris(trifluoroacetatic acid) salt (8c).* Tyrosine-derivatized resin **7c** was constructed from **6c** as described above. Cleavage of the triamine product off of the resin (0.40 g, 0.39 mmol) was accomplished using method B. In order to characterize this compound in its unprotected form, the resulting concentrated crude residue (0.175 g) was then treated with a 95 : 5 : 5 TFA : triisopropylsilane: $H_2O$ cleavage cocktail for 4 h at rt. The mixture was concentrated under reduced pressure and 20 mL of $Et_2O$ was added slowly to the residue. After precipation, the solvent was decanted off and the residue was placed under high vacuum for 12 h to give off-white solid **8c** as a free phenol (0.15 g, 66% overall crude yield). $^1$H NMR ($CD_3OD$) δ: 7.13 (d, $J$ = 8.4 Hz, 2H), 6.78 (d, $J$ = 8.4 Hz, 2H), 5.00 (br s), 3.84 (m, 1H), 3.47 (m, 1 H), 3.30-3.05 (m, 5H), 3.01 (t, $J$ = 7.6 Hz, 3H), 2.85 (dd, $J$ = 14.4 Hz, 8.7 Hz, 1H), 2.60 (m, 2H), 1.30 (t, $J$ = 7.2 Hz, 3H). $^{13}$C NMR ($CD_3OD$) δ: 158.3, 131.5, 126.1, 117.1, 58.0, 48.9, 46.8, 42.3, 37.7, 35.2, 25.3, 11.6. HRMS (ES) for $C_{14}H_{20}N_3O$ ($M^+$+H) calcd 252.207588 found 252.207932. $[\alpha]^{25}{}_D$ = (+) 6.63° (c = 6.42, MeOH).

*(4R)-t-Butoxymethyl-9-amino-3,6-diazanonane tris(trifluoroacetatic acid) salt (8d).* Serine-derivatized resin **7d** was constructed on solid support from **6d** as described above. Cleavage of the triamine product off of the resin (0.48 g, 0.39 mmol) was accomplished using method A mentioned above except there was no MeOH rinse forward, and the flask was evaporated in a cooled water bath to minimize clevage of the *t*-butoxy group. A white oily solid corresponding to **8d** was obtained (0.14g, 61% crude yield). The NMR data shows partial cleavage (5-10% of the *t*-butyl protecting group). $^1$H NMR ($CD_3OD$) δ: 3.80 (m, 3H), 3.49 (d, $J$ = 4.7 Hz, 2H), 3.40 (m, 4H), 3.07 (t, $J$ = 7.6 Hz, 2H), 2.15 (m, 2H), 1.35 (t, $J$ = 7.2 Hz, 3H), 1.23 (s, 9H); $^{13}$C NMR ($CD_3OD$) δ: 75.96, 59.37, 55.7, 47.7, 46.9, 42.2, 37.7, 27.4, 25.3, 11.4; HRMS (ES) for $C_{12}H_{30}N_3O$ ($M^+$+H) calcd 232.238888 found 232.238733. $[\alpha]^{25}_D$ = (-) 2.17° (c = 2.49, MeOH).

*(4R)-(t-Butyl)thiomethyl-9-amino-3,6-diazanonane tris(trifluoroacetatic acid) salt (8e).* Cysteine-derivatized resin **7e** was constructed on solid support from **6e** as described above. Cleavage of the triamine product off of the resin (0.46 g, 0.45 mmol) was accomplished using method B mentioned above except there was no MeOH rinse forward. A pale yellow oil corresponding to **8e** was obtained (0.20 g, 79% crude yield). $^1$H NMR ($CD_3OD$) δ: 5.05 (br s), 3.77 (m, 1H), 3.49 (d, $J$ = 5.5 Hz, 2H), 3.34 – 2.93 (m, 7H), 2.97 (dd, $J$ = 13.7, 7.1 Hz, 1H), 2.13 (m, 2H), 1.35 (t, $J$ = 7.2 Hz, 3H), 1.35 (s, 1.35, 9H); $^{13}$C NMR ($CD_3OD$) δ: 56.4, 48.9, 46.9, 44.7, 42.1, 37.7, 31.0, 28.1, 25.2, 11.5. HRMS (ES) for $C_{12}H_{30}N_3S$ ($M^+$+H) calcd 248.216045 found 248.215786. $[\alpha]^{25}_D$ = (+) 6.72° (c = 11.94, MeOH).

*(4S)-(2'-Methylthio)ethyl-9-amino-3,6-diazanonane tris(trifluoroacetatic acid) salt (8f).* Methionine-derivatized resin **7f** was constructed on solid support from **6f** as described above. Cleavage of the triamine off of the resin (0.48 g, 0.46 mmol) was accomplished using method B, except there was no MeOH rinse forward. A pale yellow oil corresponding to **7f** was obtained (0.20 g, 78% crude yield). $^1$H NMR ($CD_3OD$) δ: 4.90 (br s), 3.71 (m,1H), 3.43 (d, $J$ = 5.3 Hz, 2H), 3.31 – 3.10 (m, 4H), 3.06 (t, $J$ = 7.6 Hz, 2H), 2.65 (m, 2H), 2.12 (s, 3H), 2.16-1.98 (m, 4H), 1.34 (t, $J$ = 7.1 Hz, 3H); $^{13}$C NMR ($CD_3OD$) δ: 66.9, 55.8, 48.8, 46.9, 42.1, 37.8, 30.0,

29.3, 25.3, 15.1,11.6; HRMS (ES) for $C_{10}H_{26}N_3S$ ($M^+$+H) calcd 220.184745 found 220.185011. $[\alpha]^{25}_D$ = (+) 7.69° (c = 5.88, MeOH, turbid solution).

*(4S)-(3'-amino)propyl-9-amino-3,6-diazanonane tetrakis(trifluoroacetatic acid) salt (8i).* Glutamine-derivatized resin **7i** was constructed on solid support from **6i** as described above. Cleavage of the triamine off of the resin (52 mg, 0.044 mmol) was accomplished using method A. A pale yellow oil corresponding to **7f** was obtained (30 mg, 95% crude yield). $^1$H NMR ($CD_3OD$, 300 MHz) δ: 3.70-3.54 (m, 1H), 3.42 (d, $J$ = 5.7 Hz, 2H), 3.28-3.12 (m, 4H), 3.10-3.02 (t, $J$ = 7.6 Hz, 2H), 2.99 (t, $J$ = 6.5 Hz, 2H), 2.22-2.00 (m, 2H), 1.96-1.76 (m, 4H), 1.30 (t, $J$ = 6.9 Hz, 3H). $^{13}$C NMR ($CD_3OD$, 125.3 MHz) δ: 56.2, 47.0, 42.1, 39.9, 37.8 (2), 26.8, 25.5, 24.2, 11.6. ES-MS: m/z (intensity) 203.1 ($M^+$+H, 100), 231.1 (15), 245.1 (40). LC-ESMS: 0.1% MeCN (0.1% TFA): 99.9% $H_2O$ (0.1% TFA), 0.50 ml/min for 10 min: peak corresponding to product at 1.383 min, mass of 203.2 m/z, purity based on LC-MS 80%. HRMS (ES) for $C_{10}H_{27}N_4$ ($M^+$+H) calcd 203.223572 found 203.223771.

*(4R)-((N-t-butoxycarbonyl)-3-methylindolyl)-9-amino-3,6-diazanonane tris(trifluoroacetatic acid) salt (8j).* Tryptophan-derivatized resin **7j** was constructed on solid support from **6j** as described above. Cleavage of the triamine product off of the resin (0.46 g, 0.32 mmol) was accomplished using method A, except there was no MeOH rinse forward. A light brown solid corresponding to **8j** was obtained (0.18 g, 79% crude yield). $^1$H NMR ($CD_3OD$) δ: 8.14 (d, $J$ = 8.1 Hz, 1H), 7.71 (s, 1H), 7.64 (d, $J$ = 7.3 Hz, 2H), 7.30 (m, 2H), 5.00 (br s), 4.00 (m, 1H), 3.50 (dd, $J$ = 14.1, 7.7 Hz, 1H), 3.40 to 2.95 (m, 9H), 2.10 (m, 2H), 1.66 (s, 9H), 1.32 (t, $J$ = 7.1 Hz, 3H). $^{13}$C NMR ($CD_3OD$) δ: 150.7, 137.0, 130.8, 126.5, 125.0, 124.1, 119.8, 46.4, 42.0, 37.8, 28.3, 25.9, 25.5, 11.6; HRMS (ES) for $C_{21}H_{35}N_4O_2$ ($M^+$+H) calcd 375.276002 found 375.276561. $[\alpha]^{25}_D$ = (+) 1.62° (c = 6.56, MeOH).

*$N^{3,6,9}$-Tripropyl-12-amino-(4S)-methyl-(7S)-benzyl-3,6,9-triazaundecane tetrakis(trifluoroacetatic acid) salt (11).* To the supported borane-amine adduct **12** (obtained after reduction of 1 not subjected to any work-up, 0.250 g, 0.21 mmol, 0.84 mmol/g) swollen in DMF (4.0 mL) in a large PP vessel was added propionaldehyde (0.77 mL, 10.5 mmol) followed by vortexing for 10 minutes. At this time $NaBH(OAc)_3$ (2.44 g, 11.5 mmol) was added and the suspension was agitated for 48

hours. The suspension was then drained and washed with DMF (3×), methanol (3×), and dichloromethane (5×), and the resulting resin **10** was dried *in vacuo*. A small sample of the resin (22 mg) was then cleaved giving **11** (17 mg, 100% crude yield). HPLC-MS conditions; column: Zorbax XDB-C8 (2.1 × 50 mm, 5.0 μm); eluent: 15-85% MeCN (0.1% TFA) in water (0.1% TFA) over 2 minutes, and 85% for 4 minutes, 0.6 mL/min): single peak at 4.581 minutes corresponding to tetraamine product **11**. $^1$H NMR ($CD_3OD$, 300 MHz) δ: 7.45-7.20 (m, 5H), 3.6-2.52 (m, 20H), 2.18-1.86 (m, 3H), 1.82-1.42 (m, 4H), 1.44-1.16 (m, 9H), 1.1-0.9 (m, 7H). APT $^{13}$C NMR ($CD_3OD$, 75.5 MHz) δ: 139.6 (C), 130.5 (CH), 130.0 (CH), 129.9 (CH), 127.9 (CH), 62.2 (CH), 58.4 (CH), 54.9 ($CH_2$), 52.0 ($CH_2$), 49.9 ($CH_2$), 46.1 ($CH_2$), 39.6 ($CH_2$), 37.8 ($CH_2$), 35.7 ($CH_2$), 27.6 ($CH_2$), 26.0 ($CH_2$), 25.2 ($CH_2$), 23.1 ($CH_2$), 19.7 ($CH_2$), 19.2 ($CH_2$), 12.1 ($CH_3$), 12.0 ($CH_3$), 11.2 ($CH_3$), 11.0 ($CH_3$), 10.6 ($CH_3$). IR (MeOH cast) in $cm^{-1}$: 3300-2600 (N-H stretch), 1674.36 (C=O, TFA salt), 1202.64 (C-N stretch), 1133.99 (C-N stretch) ESMS m/z (intensity): 515.5 ($M^+$+TFA, 10), 419.5 ($M^+$+H, 100), 317.4 (B-N compound, 20). HRMS (ES) for $C_{26}H_{51}N_4$ ($M^+$+H) calcd 419.411373 found 419.410714.

*$N^{3,6,9}$-Triisobutyryl-12-amino-(4S)-methyl-(7S)-benzyl-3,6,9-triazaundecane tetrakis(trifluoroacetatic acid) salt (14).* The supported borane-adduct **12** (0.84 mmol/g resin, 0.21 mmol) was treated with isobutyraldehyde (0.96 mL 10.5 mmol) and $NaBH(OAc)_3$ (2.44 g 11.5 mmol) as described above for **11**. A small sample of the resin (24 mg) was then cleaved with 5% TFA in $CH_2Cl_2$ giving **14** (20 mg, 100% crude yield). HPLC-MS conditions; column: Zorbax XDB-C8 (4.6 × 50 mm, 3.5 μm); eluent: 25-85% MeCN (0.1% TFA) in water (0.1% TFA) over 5 minutes, and 85% for 7 minutes, 0.7 mL/min): single peak at 6.258 minutes corresponding to product **14**. $^1$H NMR ($CD_3OD$, 300 MHz) δ: 7.40-7.18 (m, 5H), 3.6-2.70 (m, 13H), 2.55-2.45 (m, 1H), 2.40-2.25 (m, 1H), 2.20-1.85 (m, 4H), 1.8-1.6 (m, 1H), 1.5-0.78 (m, 29H). APT $^{13}$C NMR ($CD_3OD$, 75.5 MHz) δ: 139.7 (C), 130.4 (2x CH), 129.9 (2x CH), 127.9 (CH), 63.1 (CH), 61.2 (CH), 60.5 (CH), 56.8 ($CH_2$), 53.3 ($CH_2$), 52.0 ($CH_2$), 46.4 ($CH_2$), 39.8 ($CH_2$), 37.9 ($CH_2$), 37.3 ($CH_2$), 28.0 ($CH_3$), 26.2 ($CH_3$), 25.8 ($CH_3$), 25.6 ($CH_3$), 25.2 ($CH_2$), 22.9 ($CH_3$), 21.3 ($CH_3$), 21.2 ($CH_3$), 21.0 ($CH_3$), 20.9

($CH_3$), 20.6 ($CH_3$), 11.7 ($CH_3$), 10.3 ($CH_3$). IR (MeOH cast) $cm^{-1}$: 3300-2600 (N-H stretch), 1674.38 (C=O, TFA salt), 1202.66 (C-N stretch), 1134.52 (C-N stretch). ES-MS m/z (intensity): 461.5 ($M^+$+H, 100), 433.5 (8), 231.4 ($M^+$+2H, 15). HRMS (ES) for $C_{29}H_{57}N_4$ ($M^+$+H) calcd 461.45823 obsd 461.457653.

*$N^6$-Methyl-12-amino-(4S)-methyl-(7S)-benzyl-3,6,9-triazaundecane tetrakis(trifluoroacetatic acid) salt (20).* The resin bound triamide **15** (0.100 g, 0.104 mmol, 0.79 mmol/g) was subjected to borane reduction and subsequent iodine work-up using the procedure described above. A small sample of the resin was cleaved with 5% TFA in $CH_2Cl_2$ giving a mixture of **19** and **20.** $^{11}B$ NMR analysis confirmed the presence of boron with a singlet at 18.31 ppm; HRMS (ES) for $C_{18}H_{34}BN_4$ ($M^+$+H) calcd 317.287653 obsd 317.288218. To 50 mg of the resin, re-suspended in THF, was added DIPEA (0.100 mL, 2 ml/g resin), ethylene glycol (0.200 mL, 4 ml/g resin). The resulting suspension was agitated for 16 hours at room temperature. The suspension was drained and washed with THF, methanol, and dichloromethane (3 times each). The resin was then dried *in vacuo* for >12h. A small sample of the resin (15 mg) was cleaved with 5% TFA/$CH_2Cl_2$ giving tetramine **20** (yellow oil, 10 mg, 100% crude yield). HPLC-MS: using the same conditions as described for **11**: major peak at 5.252 min corresponding to tetraamine product **20** (purity >80%). $^1H$ NMR ($CD_3OD$, 300 MHz) δ: 7.40-7.20 (m, 5H), 3.50 (m, 14H), 2.38 (s, 3H), 2.1 (m, 3H), 1.40-1.15 (m, 5H). APT $^{13}C$ NMR ($CD_3OD$, 125 MHz) δ: 139.4 (C), 130.2 (CH), 130.0 (CH), 127.8 (CH), 64.2 ($CH_3$), 58.4 ($CH_2$), 53.7 (CH), 46.2 ($CH_2$), 41.2 ($CH_2$), 38.0 ($CH_2$), 37.7 (CH), 32.4 ($CH_2$), 25.2 ($CH_2$), 14.4 ($CH_3$), 11.5 ($CH_3$). ES-MS m/z (intensity): 307.3 ($M^+$+H, 100). HRMS (ES) for $C_{18}H_{35}N_4$ ($M^+$+H) calcd 307.286172 obsd 307.286305.

*N-Ethyl-L-phenylalanine methyl ester (22).* A solution of borane-tetrahydrofuran (1M, 10.0 mL, 10.0 mmol) was added dropwise to a solution of amide **21** (1.0 g, 4.5 mmol) in dry tetrahydrofuran (10 mL) maintained at 0°C. The resulting solution was warmed up to room temperature, then heated at 65 °C for 3 hours, then cooled back to rt. Triethylamine (2.0 mL), glacial acetic acid (3.0 mL), and iodine (1.25 g, 5.0 mmol, dissolved in 5 mL THF) were successively added. The mixture was stirred for 1 h, after which time a solution of aqueous sodium hydroxide (1M, 100 mL) was slowly added, followed by aqueous saturated sodium thiosulfate (10 mL). The

resulting mixture was extracted with diethyl ether (2 × 50 mL) and ethyl acetate (2 × 50 mL). The combined organic layers were washed with brine (25 mL), dried over anhydrous magnesium sulfate, evaporated, then dried under high vacuum. Crude amine **22** was obtained as an oil (0.85 g, 86%). $^1$H NMR (300 MHz, $CDCl_3$) δ: 7.40-7.15 (m, 5H), 3.63 (s, 3H), 3.55 (t, $J$ = 7.0 Hz, 1H), 2.97 (AB m, 2H), 2.70-2.45 (AB m, 2H), 1.75 (br s, 1H), 1.07 (t, $J$ = 7.0 Hz, 3H). $^{13}$C NMR (75 MHz, $CDCl_3$) δ: 174.9 (C), 137.1 (C), 128.9 (CH), 128.2 (CH), 126.5 ( CH), 62.7 ($CH_3$), 51.3 (CH), 42.2 ($CH_2$), 39.5 ($CH_2$), 15.0 ($CH_3$). ES-MS m/z (intensity): 208.1 ($M^+$+H, 100).

*N-Benzyl butyramide (23).* To a solution of benzylamine (6.0 mL, 0.055 mol) and triethylamine (15.2 mL, 0.110 mol) in anhydrous DMF (50 mL) was slowly added butyric anhydride (44.7 mL, 0.275 mol). The solution was stirred for 5 h at room temperature after which time it was reduced to half volume on a rotary evaporator. Water (50 mL) was added and the mixture was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with 10% aqueous acetic acid (3 × 30 mL), aqueous saturated sodium bicarbonate (3 × 30 mL), brine (3 × 30 mL), and dried over anhydrous magnesium sulphate. The solution was evaporated and the resulting light beige solid was dried under high vacuum for > 12 h (7.16 g, 74%). $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.40-7.25 (m, 5H), 5.70 (br s, 1H), 4.21 (d, $J$ = 6 Hz, 2H), 2.20 (t, $J$ = 7.5 Hz, 2H), 1.70 (m, 2H), 0.96 (t, $J$ = 7.5 Hz, 3H).

*N'-Butyl-N-benzylamine (26).* A solution of borane-tetrahydrofuran (1M, 2.50 mL, 2.50 mmol) was added dropwise to a solution of amide **23** (2.0 g, 11.3 mmol) in dry tetrahydrofuran (2.5 mL) at room temperature. The resulting solution was heated at 65 °C for 12 hours, then cooled to room temperature. Methanol was added (1.0 mL) to neutralize excess borane. Then, a 4M solution of HCl/dioxane (0.60 mL) was added slowly and stirred for 2.5 h. Aqueous sodium hydroxide (1M, 15 mL) was added and the mixture was extracted with diethyl ether (3 × 15 mL). The combined organic layers were dried over anhydrous magnesium sulfate, evaporated, and dried under high vacuum for no longer than 15 minutes in order to minimize evaporation of the amine product. Crude amine **26** was obtained as a clear oil in essentially pure form (1.5 g, 81%). $^1$H NMR (300 MHz, $CDCl_3$) δ: 7.4-7.2 (m, 5H),

3.80 (s, 2H), 2.60 (t, $J = 7$ Hz, 2H), 1.5 (m, 2H), 1.3 (m, 2H), 0.95 (t, $J = 7$ Hz, 3H). $^{1}$H NMR (300 MHz, $CD_3OD$) δ: 7.4-7.2 (m, 5H), 3.85 (s, 2H), 2.72 (m, 2H), 1.50 (m, 2H), 1.36 (m, 2H), 0.92 (t, $J = 7.5$ Hz, 3H).

*Borane-amine adduct 25.* Under a nitrogen atmosphere, a solution of borane-tetrahydrofuran (1M, 53 μL, 0.053 mmol) was added slowly to a solution of amine **26** (79 mg, 0.49 mmol) in tetrahydrofuran (1.0 mL) at room temperature. After 2 hours, excess borane was neutralized by addition of a solution of methanol-tetrahydrofuran (1:1, 4 mL). The resulting solution was evaporated and dried under high vacuum to provide **25** as a white solid (85 mg). The chiral adduct shows several diastereotopically distinguished hydrogens by proton NMR. $^{1}$H NMR (300 MHz, $CDCl_3$) δ: 7.45-7.2 (m, 5H), 4.20 (dd, $J = 14$ Hz, 4 Hz, 1H), 3.66 (dd, $J = 14$ Hz, 9 Hz, 1H), 3.35 (br s, 1H), 2.68 (m, 2H), 1.68 (m, 1H), 1.58 (m, 1H), 1.19 (m, 2H), 2.0-1.3 (br s, 3H, $-BH_3$, merges as a sharp singlet (1.70 ppm) upon $^{1}H[^{11}B]$ decoupling)), 0.80 (t, $J = 8$ Hz, 3H). $^{1}$H NMR (300 MHz, $CD_3OD$) δ: 7.45-7.2 (m, 5H), 4.05 (d, $J = 10$ Hz, 1H), 3.55 (d, $J = 10$ Hz, 1H), 2.58 (m, 1H), 2.43 (m, 1H), 1.66 (m, 1H), 1.53 (m, 1H), 1.18 (m, 2H), 2.0-1.3 (br s, 3H, $-BH_3$), 0.83 (t, $J = 7$ Hz, 3H). $^{11}B\ [^{1}H]$ NMR (128.4 MHz, $CD_3OD$) δ: - 14.9 (q, $J = 90$ Hz). APT $^{13}$C NMR (75 MHz, $CDCl_3$) δ: 134.5 (C), 129.3 (CH), 129.2 (CH), 128.9 (CH), 60.1 ($CH_2$), 53.2 ($CH_2$), 28.5 ($CH_2$), 19.9 ($CH_2$), 13.5 ($CH_3$).

*Control experiments for iodine equivalence.* A sample of amide **23** (0.10 g, 0.57 mmol) was reduced with borane-tetrahydrofuran (1M, 1.30 mL, 1.30 mmol) in tetrahydrofuran (1.3 mL) as described above for the preparation of **26**. The mixture was stirred at 65 °C for 12 hours after which time it was cooled to room temperature. Diisopropylethylamine (0.75 mL) and glacial acetic acid (1.5 mL) were successively added, followed by the necessary amount of iodine indicated in Table 2 (as a concentrated THF solution). The solution was stirred for 3 hours then aqueous saturated sodium thiosulphate (10 mL) and aqueous saturated sodium bicarbonate (15 mL) were added. The mixture was extracted with diethyl ether (3 × 15 mL) and the combined organic layers were dried over anhydrous magnesium sulphate, filtered and evaporated, then dried under high vacuum for 15 minutes. The relative ratio of **25** and

26 was quantified by $^1H$ NMR analysis (integration and peak heights from representative signals).

**Table 2. Assays toward mechanistic studies.**

| entry | conditions[a] | temp. (°C) | time (h) | ratio 25:26 (%)[b] |
|---|---|---|---|---|
| 1 | acidic work-up (aq. 1N HCl) | rt | 2 | 0:100 |
| 2 | no work-up | rt | 2 | 80:20 |
| 3 | 1:2:7 DIPEA/AcOH/THF | rt | 2 | 60:40 |
| 4 | same solvent, 0.5 equiv. $I_2$ | rt | 2 | 30:70 |
| 5 | same solvent, 1.0 equiv. $I_2$ | rt | 2 | 0:100 |
| 6 | MeOH/THF | rt | 2 | 80:20 |
| 7 | 1:2:7 DIPEA/MeOH/THF | rt | 2 | 80:20 |
| 8 | same solvent, 1.0 equiv. $I_2$ | rt | 3 | 75:25 |
| 9 | excess TFA, THF | rt | 2 | 0:100 |
| 10 | aq. conc. NaOH, THF | rt | 20 | 75:25 |
| 11 | 1:9 DIPEA/ THF | rt | 2 | 85:15 |
| 12 | morpholine (excess), THF | rt | 2 | 0:100 |

[a] General conditions: model amide **23** was reduced with 2.2 equiv. $BH_3$, 65 °C, 3-4 h; then the indicated work-up is applied , followed by addition of aqueous base and thiosulfate (if necessary), followed by extractions with diethyl ether. [b] Measured by comparison of integrals of representative signals from the $^1H$ NMR spectra of the crude reaction mixture (estimated error: 5%).

*Conclusions.* In summary, described in this Example 1 is an exemplary method of the invention, a mild work-up protocol for the cleavage of borane-amine adducts arising from the reduction of polyamides supported onto water-compatible trityl-based resins. It was demonstrated that chiral polyamines with diverse side chain functionalities can be generated as free bases without premature release from the solid support, and with essentially no racemization using the method of the invention. When subjected to this method, supported oligomeric secondary amides **6** provided triamine products **8** in high yields and good to excellent purity. Substrates such as **15** containing a tertiary amide required more stringent work-up conditions to liberate the polyamine product **20**. Model studies carried out in solution with model amide **23** confirmed the efficiency of the buffered iodine solution, and highlighted several

advantages (no heating necessary, no need for strong bases or acids) of the invention over existing methods for the cleavage of borane-amine adducts. Although the use of excess iodine is recommended in solid-phase applications, control solution-phase experiments revealed that only one equivalent of iodine is necessary to effect complete cleavage of borane-amine adducts. The acetic acid-acetate buffer also plays a crucial role since its replacement with methanol was found ineffective. Consequently, described herein is a proposed mechanism involving all components (iodine, acetic acid and acetate from the buffer system) in which borane-amine adducts are transformed first to the monoiodoborane-amine, then to the corresponding acetoxyborane-amine adduct of much weaker coordination affinity. The latter would dissociate readily and get trapped by excess acetic acid to provide the desired secondary amine in its protonated form.

Additional requirements encountered in solid-phase synthesis, such as the need to attach the substrate to a linker that resists subsequent transformations and can be cleaved selectively at the end, are provided by the effective and milder reaction conditions of the invention. In turn, these methods can find use in solution-phase synthesis. In addition to showing that this new iodine-promoted work-up is useful in the reduction of amides in solution, control experiments demonstrated that the buffered iodine solution is truly effective for cleaving borane-amine adducts, as opposed to the trifluoroacetic acid employed in the resin cleavage operation. Therefore, this reduction/oxidative work-up protocol is not limited as a general method for the synthesis of polyamines to be released and employed in solution. It is also useful toward the screening of bead-supported libraries of oligoamine derivatives assembled through split-pool synthesis (for an example of screening bead-supported polyamine libraries against polyanionic compounds, the entire contents of which are incorporated herein by reference, see, Manku, S.; Hall, D. G. *Org. Lett.* **2002**, *4*, 31-34.).

**Example 2: Screening Bead-Supported Polyamine Libraries Against Polyanionic Compounds**

In spite of their ubiquity and their essential roles in living systems, only a small number of distinct polyamines exist in nature[1].[2,3] Notable examples of these vital

biomolecules include putrescine, spermidine, and spermine. Polyamines are protonated under physiological conditions and are thus predisposed to form strong salt bridges. These cationic molecules were shown to condense with the phosphate groups of DNA and RNA,[4] with anionic oligosaccharides,[5] and with the carboxylate side chains of aspartate and glutamate residues in polypeptides[6] and proteins.[7] Yet, it remains to be seen whether the fine structure of the interammonium spacers in polyamines can be tuned to optimize binding affinity and selectivity in multipoint ion-pairing complexes in aqueous media.[8,9] Nature provides relatively few insights to address this question; the main biogenic polyamines, made almost exclusively with 1,3-diaminopropyl and 1,4-diaminobutyl units, display little diversity in their sequence and length as compared to the other natural biopolymers (peptides, nucleic acids, and oligosaccharides). By way of multivalent ion-pairing, we hypothesize that large combinatorial libraries of unnatural polyamines could reveal highly potent and selective ligands for polyanionic biomolecules (Figure 22). As a first step towards this goal, we describe the first demonstration of on-bead screening in mixed aqueous-organic media, using an encoded structural library of unnatural polyamines synthesized by a split-pool approach.[10]

As a proof of principle a relatively small prototypical library of polyamines was synthesized and screened against model polyanionic molecules. The trisulfonated dyes 1 and 2 were selected as examples of rigid targets for multivalent ion-pairing (Figure 23).[11] They are also reminiscent of the polysulfated heparin oligosaccharides and thus serve as models for the eventual development of ligands and molecular sensors for these biologically important carbohydrates.[12] From a practical standpoint, these red-colored dyes also provide a direct means of visually detecting bead hits in the screening experiments. In addition, being rather amphiphilic, they are soluble in mixed aqueous organic solvents, and thus are appropriate for screening against a library of polyamines supported onto polystyrene-based supports.

To access the library, the synthetic plan was centered on a "library from library" approach[13] whereby a split-pool library of polyamide precursors was exhaustively reduced to polyamines using the borane promoted procedure for peptides supported onto a trityl linker (Figure 22).[14] Library design was based on optimizing

structural diversity with a limited but representative set of 14 amino acid building blocks offering a variety of geometrical features (length and flexibility) (Figure 24). Split-pool libraries of end-acetylated di- and tripeptides were assembled using Fmoc-amino acid coupling methods from a trityl bound dodecamine spacer.[15] Such a long spacer was selected in order to avoid steric interference from the triphenyl moiety. The library was encoded by termination synthesis whereby 10% of the corresponding N-acetyl or N-butyryl aminoacids were employed in each coupling step.[16] This method, which eventually allows unambiguous identification of the oligoamines by electrospray mass spectrometry (ES-MS), can even distinguish between isobaric sequences by "ladder" decoding of truncated fragments.[17]

Exhaustive reduction of the tripeptide library provided the 2744-membered tetraamine library. The corresponding 196-membered library of triamines was also made. Over 20 beads from the larger tetraamine library were picked at random in order to test the validity of the synthetic approach and the encoding method. The beads were cleaved individually in microvials with 5% TFA/$CH_2Cl_2$, and the resulting material was analyzed by LC-ES-MS. From these test runs and other subsequent ones, decoding efficiencies in the 85-95% range were routinely obtained (see examples in Supporting Information).

The N-ethyl-terminated triamine library $\{-(CH_2)_{12}NH\text{-}R^1\text{-}NH\text{-}R^2\text{-}NHCH_2CH_3\}$ was employed for this study since the model sulfonated targets are triply anionic species. The triamines are not quite symmetrical, the two end-groups presenting slightly different degrees of size and hydrophobicity. Control experiments (with and without 0.1% Triton X-100) with the uncharged tripeptide library confirmed the absence of non-specific interactions between the dyes and the polymer matrix. Thus the triamine library (3 mg, approx. $10^4$ beads), in a large stoichiometric excess, was screened against 39 μM dye 1 in 0.4 mL 10% aqueous 50 mM TRIS-MES buffer (pH 7.0) in DMF. These conditions provide a huge excess of water molecules vis-à-vis the target dye. A proportion of about 50-70% of all beads became distinctly pink, of which approximately 5-6% had a deeply red-colored appearance. Decoding results for the darkest beads are shown in Table 3 as a function of single residue frequency with respect to their position within the triamine backbone ($R^1$, $R^2$).

**Table 3. Screening results between triamine library and dye targets 1 and 2 expressed in residue occurrence per position.[a]**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| dye | | 1 | 1 | 1 | 2 | 2 | |
| pH | | 7.0 | 7.0 | 5.5 | 7.0 | 5.5 | |
| additive[b] | | - | SP | SP | SP | SP | |
| # beads | | 30 | 26 | 23 | 40 | 31 | |
| position[c] | | 1, 2 | 1, 2 | 1, 2 | 1, 2 | 1, 2 | |
| $12Abo^R$ | | 8, 4 | 9, 8 | 14, 9 | 3, 5 | 5, 6 | |
| $8Aoc^R$ | 10, 13 | 7, 14 | 1, 3 | 18, 5 | 5, 2 | | |
| $6Ahx^R$ | 2, 5 | 3, 0 | 1, 1 | 9, 6 | 7, 3 | | |
| $\gamma Abu^R$ | 0, 1 | 0, 0 | 0, 0 | 0, 3 | 0, 0 | | |
| $\beta Ala^R$ | 1, 1 | 1, 2 | 0, 1 | 1, 3 | 0, 0 | | |
| $Gly^R$ | | 0, 0 | 0, 0 | 0, 0 | 0, 1 | 1, 0 | |
| $LNva^R$ | 0, 0 | 0, 0 | 0, 2 | 0, 1 | 0, 1 | | |
| $DNva^R$ | | 0, 0 | 0, 0 | 0, 1 | 0, 1 | 1, 3 | |
| $LPhe^R$ | 0, 0 | 0, 0 | 0, 2 | 0, 1 | 0, 0 | | |
| $DPhe^R$ | 0, 0 | 0, 0 | 2, 0 | 0, 1 | 0, 2 | | |
| $2Acc^R$ | 0, 1 | 0, 0 | 0, 0 | 4, 2 | 8, 3 | | |
| $4Acc^R$ | 2, 3 | 5, 0 | 4, 4 | 2, 4 | 4, 3 | | |
| $4Amc^R$ | | 7, 2 | 1, 2 | 0, 0 | 3, 6 | 0, 6 | |
| $4Amb^R$ | | 0, 0 | 0, 0 | 1, 0 | 0, 1 | 0, 2 | |
| Most | | $8Aoc^R$ $8Aoc^R$ | | $12Abo^R$ | $8Aoc^R$ | $12Abo^R$ | |
| Frequent | | $12Abo^R$ | | $12Abo^R$ | $4Acc^R$ | $6Ahx^R$ | $2Acc^R$ |
| Residues[d] | | $4Amc^R$ | | | $4Amc^R$ | $6Ahx^R$ | |
| | | $6Ahx^R$ | | | $12Abo^R$ | $8Aoc^R$ | |
| | | | | | $2Acc^R$ | $4Acc^R$ | |

[a] The R superscript indicates a reduced amino acid residue. Each assay was repeated once and was shown reproducible. [b] SP = spermidine. [c] Positions 1 and 2 refer to the interammonium spacers $CH_2R^1$ and $CH_2R^2$ respectively (Figure 1). [d] See Supporting Information Section for full sequence results.

Out of 30 of the darkest beads picked under a microscope (1st column), most had triamines with either the long twelve and eight carbon interammonium spacers $12Abo^R$ and $8Aoc^R$ regardless of the position. For instance, some of the most recurrent sequences were $12Abo^R$-$8Aoc^R$ and $8Aoc^R$-$8Aoc^R$ (found four times each). A small

number of beads also had the relatively long but more rigid spacers $6Ahx^R$ and $4Acc^R$. The assay was repeated with 0.3 mM spermidine (2nd column) as a competitive ligand to minimize non-specific ionic interactions, and also at pH 5.5 (3rd column), a precautionary measure to ensure that even short triamines with the two-carbon interammonium spacers from α-amino acids were largely protonated.[18] Binding did not seem affected and the overall selectivity was conserved.[19] Screening of dye 2 provided similar residue consensus (4th and 5th columns), however reduced in $12Abo^R$ to include more $6Ahx^R$, and also $2Acc^R$ as a frequent residue that was essentially absent from the screening of dye 1. Aside from the remarkable degree of selectivity observed, an interesting feature of these results is the almost total absence of $\beta Ala^R$ and $\gamma Abu^R$, the respective 3-carbon and 4-carbon interammonium spacers found in the natural polyamines. The presence of 1:1 binding stoichiometry in this system was assumed based on a Job's plot obtained from NMR titration[20] performed between model triamine $MeCONH(CH_2)_6$-NH-$8Aoc^R$-$8Aoc^R$-Et and dye 1.[21] This allows a tentative rationalization of sequence selectivity based on triply ion-paired complexes involving a combination of electrostatic and hydrogen bonding interactions. Hydrophobic interactions do not seem dominant since exposure of the same dye to the precursor peptide library, and to the triamine library in its neutral form (at pH 11), provided only faint beads. Except for rotation along the N-naphthyl bonds, the framework of dyes 1 and 2 is almost entirely rigid. From inspecting hand held models we have identified two reasonable planar conformers for each dye (Figure 25).

As shown for dye 1, in both conformers A and B the sulfonate groups are relatively far apart. The meta sulfonates of dye 2, however, are much closer spatially. The relative positioning for the three sulfonate groups in 2 is identical for both conformers. As a basis for guiding comparisons, all indicated distances were measured between farthest oxygens of these groups on minimized structures.[22] Similarly, internitrogen distances were calculated for the most frequent residues in their extended conformation: $12Abo^R$ (16.6 Å), $8Aoc^R$ (11.5 Å), $6Ahx^R$, (8.9 Å), 4Amc (7.2 Å), $4Acc^R$ (5.4 Å), $2Acc^R$ (4.5 Å). This simple analysis accounts especially well for the high preference for $8Aoc^R$, $6Ahx^R$, and $4Acc^R$ in the case of both dyes, and for $2Acc^R$ as a shorter, geometrically suitable spacer for bridging the meta sulfonates in dye 2.

The presence of $12Abo^R$ as one of the preferred residues, in particular with 1, could be accounted by two-point binding across farthest sulfonate groups. Overall, the most favored dye-triamine complexes appeared to be tightly bound, as shown by the $K_a$ value of 6400 $M^{-1}$ measured between model compound $MeCONH(CH_2)_6$-NH-$8Aoc^R$-$8Aoc^R$-Et and dye 1.[19,23]

Most interestingly, the results of Table 3 are useful in the context of designing target-selective triamine ligands. This was demonstrated by the design of $2Acc^R$-$6Ahx^R$, a sequence foreseen as a specific ligand for dye 2 after comparing triamine residue consensus for 1 and 2, and from the measurement of $K_a$ values using $MeCONH(CH_2)_6$-NH-$2Acc^R$-$6Ahx^R$-Et as model ($K_a$ with dye 1 = 1100 $M^{-1}$; $K_a$ with dye 2 = 2700 $M^{-1}$)[19,22]. In a "fishing-out" experiment, a mixture of these two dyes (1:1 ratio) was incubated with resin-bound $-(CH_2)_{12}$-NH-$2Acc^R$-$6Ahx^R$-Et triamine as described above (pH 7.0, 50 mM MES-TRIS $H_2O$/DMF 1:9). The beads, which became red instantaneously, were rinsed several times with a 0.1 M DMF solution of $PhSO_3Na$, then with a 0.5 M solution in order to elute off the bound dye. As shown in the HPLC chromatogram of the final fraction (Figure 26), dye 2 was detected with approximately 97% purity, thereby confirming that highly target-selective polyamines can be designed from a library approach. This work on bead-supported encoded libraries of unnatural polyamines shows that a combinatorial approach, even with a relatively small library of linear flexible polyamines, can provide structural selectivity in multivalent ion-pairing in mixed aqueous-organic media, and even turn in target-selective ligands. The elaboration of larger polyamine libraries on fully hydrophilic supports will help in the discovery of ligands and sensors for polyanionic biomolecules.

**Example 2: Preparing Combinatorial Libraries of Resin-Conjugated Polyamines**

The following example describes exemplary protocols for practicing the methods of the invention to prepare combinatorial libraries of "exo-peptides" or polyamines using hydrophilic triarylmethyl resins. An exemplary "exo-peptide" library of the invention having with up to 10,368 rotamers was synthesized.

All reagents used are commercially available and were employed without further purification. Fmoc-, or Ac-protected and free amino acids, PS-Trityl

chloride resin (200-400 mesh, 1% DVB, 0.80 mmol/g) and TentaGel S Br resin as well as ArgoGel Cl were purchased from Novabiochem (San Diego, California), Advanced Chemtech (Louisville, KY), Rapp-Polymere ( Tuebingen, Germany) and Argonaut Technologies Inc. ( San Carlos, USA ) respectively. Chlorotriarylmethane conjugated PEG-PS resin was prepared. All glassware used in solid-phase reactions had been silanized by treatment with 20% chlorotrimethylsilane/toluene for 12h and then dried under vacuum. Polypropylene(PP) filter vessels were obtained from Bio-Rad. THF was dried by distillation over sodium/benzophenone ketyl, $CH_2Cl_2$ over sodium hydride. Anhydrous DMF was obtained commercially from Aldrich. NMR spectra were recorded at Bruker AM-360. Low and high resolution ES-MS were done on a Hewlett-Packard 1100 MSD and ZabSpecETOF respectively. Libraries were decoded by RP-HPLC-MS or ES-MS on a Hewlett-Packard 1100 system using conditions described in the manuscript. All reactions involving TentaGel and ArgoGel resins were gently stirred or vortexed. Abbreviations: HBTU – 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyl-uronium hexa-fluorophosphate, HOBt – N-Hydroxybenzo-triazole, DIPEA – Diisopropylethyl-amine, TFA – Trifluoroacetic acid.

*General Process for the preparation of hydrophilic triarylmethyl resins of the present invention*

As a first step in making the"exo-peptide" library, a "chlorotriarylmethane conjugated PEG-PS resin 30was generated using a strategy of coupling the commercially available TentalGel (or ArgoGel) resin with a trityl group through an $-OCH_2CH_2$- linker, as illustrated in Figure 14. This resin possesses a good compatibility with the $BH_3/I_2$ reduction procedure and a reasonably high loading (~0.24 mmol/g). The model polyamine **32** (as illustrated in Figure 15A) was synthesized by reducing its precursor, chlorotriarylmethane conjugated PEG-PS resin-bound polyamide **31** using the $BH_3/I_2$ reduction procedure of the invention (as described above).

In another embodiment of the synthesis, Tentagel MB Br (250 μm beads) was used. Reaction time of the Tentagel with the benzophenone compound was 5 days. Reaction of the resin-conjugated benzophenone **27** with an acid was 8 hours to form **28**. Reaction of 28 with an arylmagensium halide took place for an extended

period of time (.e.g, overnight). In one embodiment, conversion of **29** to **30** with thionyl chloride was carried out in two additions at approximately 2 hours and 3 hours. In other embodiments, 30 is reacted with a spacer such as $NH_2(CH2)_{12}NH_2$ upon portionwise addition to the diamine over a period of time, e.g., 1 hour and vortexed overnight.

**Example: Preparation of Grignard reagent and the resin:**

*1. Preparation of acetal protected benzophenone:*

4-bromobenzophenone (13.06 g, 50 mmol), ethylene glycol (3.41 g, 3.06 cm3, 55 mmol) and 4-toluenesulfonic acid (190 mgs, 1 mmol) were dissolved in benzene (30 cm3) and refluxed for 4 days under Dean-Stark conditions. After this time, the reaction mixture was poured onto saturated aqueous NaHCO3 solution, extracted with ether (2 x 50 cm3), washed (sat. aqueous NaHCO3 and brine), dried (MgSO4) and concentrated under reduced pressure to afford the product as an off-white powder (13.9g, 90%). The crude product is then stored overnight in a desiccator, under vacuum, over P2O5. The product is then used without further purification.

2. *Preparation of Grignard reagent*:

To magnesium metal (126 mgs, 5.2 mmol) and iodine (1 crystal) stirring under an inert atmosphere in dry THF (20 cm3) was added a solution of the protected bromobenzophenone (1.74 g, 5.7 mmol) in THF. The resulting solution was then stirred at reflux until all the magnesium had reacted. The solution of the Grignard reagent was then taken on to the next step.

*3. Addition to the Bromo-Tentagel resin:*

TentaGel MB Br resin (Rapp-Polymere, 0.26 mmol g-1 Br, 500 mgs, 0.13 mmol) and CuBr×SMe2 (53 mgs, 0.26 mmol) were suspended in dry THF (7 cm3) and gently stirred under an inert atmosphere. Distilled HMPA (2 cm3) was then added to the stirring suspension, followed by the solution of the Grignard reagent prepared previously (20 cm3, 5.2 mmol). The mixture was then stirred at 65°C for 5 days. After this time, the reaction was quenched by the addition of saturated aqueous NH4Cl and transferred to a 70 cm3 PP vessel. The suspension was then filtered and washed successively with sat. aqueous NH4Cl, H2O, H2O/THF (1:1) methanol and

dichloromethane (4 times each). The resin is first dried at the pump, then under high vacuum overnight.

*4. Removal of the acetal:*

Resin from previous step 3.) (445 mgs) was suspended in a mixture of perchloric acid and dichloromethane (1:10, 15cm3), in a 70 cm3 PP vessel. The PP vessel was then sealed and placed on the wrist shaker at a speed just high enough to achieve sufficient agitation of the resin beads. The sample was shaken overnight, then filtered. The resin was then washed with H2O/THF (1:1), DMF/NEt3 (3:1), methanol and dichloromethane (4 times each). The resin was first dried at the pump, then under high vacuum overnight.

*5. Preparation and addition of phenylmagnesium Grignard to the free resin-supported benzophenone.*

To magnesium metal (63 mgs, 2.6 mmol) and iodine (1 crystal) stirring under an inert atmosphere in dry THF, cooling in an ice bath was added distilled bromobenzene (455 mgs, 305 mm3, 2.9 mmol) slowly over a period of 10 minutes. The solution was then stirred until all the magnesium had reacted. The Grignard reagent was then taken onto the next step in the synthesis.

To the resin, suspended in dry THF (10 cm3) was added the preformed solution of phenylmagnesium bromide in THF. The reaction mixture was then stirred at room temperature for 7 hours under an inert atmosphere. After this time, the reaction was quenched by the addition of 0.5M HCl solution (5 cm3). The resin was transferred to a 70 $cm^3$ PP vessel and then washed successively with H2O, 0.5M NaHCO3 solution, H2O, H2O/THF (1:1), methanol and dichloromethane (3 times each). The resin was then dried under high vacuum overnight.

*6. Activation of the hydroxy-trityl resin into the chlorotrityl resin:*

The resin is suspended in dry dichloromethane (10 $cm^3$) in a 70 $cm^3$ PP vessel and vortexed gently. To the suspension is then added thionyl chloride (1$cm^3$). The resulting suspension is then vortexed for a further 30 minutes. The suspension is filtered, and the resin resuspended in dry dichloromethane. Thionyl chloride is again added, and the PP vessel vortexed for 2 hours. The resin is then filtered, washed with dry dichloromethane and dried under high vacuum overnight.

*7. Attachment of a diamine spacer to the chlorotrityl resin:*

4,7,10-trioxa-1,13-tridecanediamine (3 $cm^3$, 13.6 mmol) was dissolved in dry dichloromethane (15 $cm^3$) in a 70 cm3 PP vessel. To the solution was added the resin in 4 portions over 30 minutes. After each addition the PP vessel was vortexed gently. After the addition of all the resin to the solution, the suspension was vortex overnight. After this time, the resin is filtered and then washed successively with methanol, DMF/NEt3 (4:1), methanol and dichloromethane (4 times each). The resin is then dried under high vacuum overnight.

*8. Example of coupling of a first Fmoc-aminoacid:*

Resin (519 mgs, approx. 0.1 mmol) and Fmoc-4-aminomethyl benzoic acid (149 mgs, 0.4 mmol) were weighed into a 20 $cm^3$ PP vessel. To this was added anhydrous DMF (5 $cm^3$) and the resulting suspension was vortexed for 5 minutes. HOBt (61 mgs, 0.4 mmol) and HBTU (152 mgs, 0.4 mmol), dissolved in DMF (5 $cm^3$) were then added to the suspension and the vortexing continued for 2 minutes. *N,N*-diisopropylamine (52 mgs, 70mm3, 0.8 mmol) was then added to the suspension and vortexing continued for 5 hours. The resin was then filtered and washed with DMF, methanol and dichloromethane (3 times each).

*9. Example of deprotection of Fmoc group:*

To the resin in a 20$cm^3$ PP vessel was added a 30% solution of piperidine in DMF (5 $cm^3$). The suspension was then vortexed gently for 10 minutes. The resin was filtered, and then resuspended in the cleavage solution. The suspension was then vortexed for a further 1 hour, and the resin then filtered. The resin was washed with DMF, methanol (3 times each) and dichloromethane (5 times).

*10. Example of borane reduction with piperidine workup:*

Resin (approx. 1.0 g, 0.25 mmol) is weighed into a flask fitted with a condenser, under an inert atmosphere (NOTE: no stirrer bar present). The resin is swollen and suspended in dry THF (10 $cm^3$) and to this is added borane-tetrahydrofuran complex (1M solution in THF, 5 cm3, 5 mmol). The reaction mixture is then heated at 65*f*C for between 2 and 3 days. The resin is then filtered, washed with methanol, and then resuspended in piperidine (10-15 $cm^3$). The suspension is again heated at 65°C for 24

hours. The resin is then filtered and washed with THF, methanol and dichloromethane (3 times each). The resin is then dried under high vacuum.

*An exemplary procedure for reduction of polyamide 31 to model polyamine 32 with the $BH_3/I_2$-method.* Chlorotriarylmethane conjugated PEG-PS resin-bound polyamide **31** (Figure 15A) (200 mg, 0.048 mmol, 0.24mmol/g) was weighed into a 10 mL silanized round bottom flask and swelled in dry THF (0.5 mL) under nitrogen. The diborane solution (1M in THF, 2.9 mL, 2.9 mmol) was added dropwise at RT over 2 min. The flask was then equipped with a condenser and the suspension was gently refluxed at 65°C for 72h. Upon cooling to RT, the suspended resin was rapidly transferred into a PP filter vessel via a silanized pipette using dry THF to rinse out the flask and to wash the resin extensively. Then, dry THF (0.3 mL), anhydrous DIPEA (0.08 mL) and $EtCO_2H$ (0.16 mL) were added successively. After shaking the suspension the iodine was added (0.38g, 1.5 mmol in 0.66 mL THF) and the vessel was vortexed for 4h. The resin was then filtered and rinsed with THF, 1:3 $Et_3N$/DMF, MeOH and $CH_2Cl_2$ (3 times each) and dried under high-vacuum overnight to give the resin-bound polyamine **7** (~190 mg). Its ES-MS gave the excellent result and a "+24" peak was hardly observed (in some cases an peak ($MH^+$+24) appeared in the polyamine **7**'s ES-MS spectrum, as discussed in further detail, below).

*Library synthesis:*

1) Synthesis of 12 encoding compounds – general

*Method 1* : To solution of an enantiomerically pure amino acid (1.0 mmol) in 3 mL of 2 M NaOH:THF (1:1) was added butyryl chloride (0.21 mL, 2 mmol) or heptanoyl chloride (0.31 mL, 2 mmol). The mixture was stirred at RT for 5h, which pH value was then adjusted to 5 by 1 M HCl, extracted by $CH_2Cl_2$ (8mLX3). The organic layer was dried with brine and $MgSO_4$, and concentrated. The product was further dried under vacuum for a few days, which was confirmed by $^1$H-NMR. (See Nakanishi (1997) Bioorganic & Medicinal Chemistry 5:1969-1988).

*Method 2*: An enantiomerically pure amino acid (2 mmol) was added to ice-cold 1 M NaOH (2 mL) and the stirred solution was cooled in an ice-water bath. Chilled 1 M NaOH (0.4 mL) was added followed by acetic anhydride (40 µL, 0.4 mmol). The reaction of the solution should be distinctly alkaline, if necessary a small

volume of 1 M NaOH was added to assure alkalinity. The addition of 1 M NaOH and acetic anhydride (the same amounts) was repeated four more times with testing for alkalinity and adjustment, if necessary, after each addition. Stirring was then continued for 30 min. The solution was acidified to Congo with concentrated hydrochloric acid, and extracted with $CH_2Cl_2$, dried over $MgSO_4$ and evaporated. The product was dried under vacuum for a few days, which was confirmed by $^1$H-NMR. (see Bodanszky, M. and Bodanszky, A., 2nd Ed. *The Practice of Peptide Synthesis*, Springer-Verlag: Berlin Heidelberg, 1994, pp186-188).

2) General procedure for the first amino acid coupling

*$NH_2$-Functionalized resin* **31** (see Figure 16): 4,7,10-Trioxa-1,13-tridecanediamine (97%, 15 mL, 66 mmol) was dissolved in 15 mL of a dry CHCL in a silanized flask. Chlorotriarylmethane conjugated PEG-PS resin (~3.7g, estimated loading: ~0.33 mmol/g) was then added to the solution in 4 portions over one hour with stirring in between additions. After stirring for an additional hour, 5 mL of methanol was added followed by another 20 min. of stirring. The resin was transferred into a PP filter vessel. It was then filtered and rinsed with MeOH, 1:3 $Et_3N$/DMF, MeOH and $CH_2Cl_2$ (4 times each), and dried under high-vacuum for over 24 hours to give $NH_2$-functionalized resin **8** (3.8g, 0.24 mmol/g average, Fmoc release U.V. assay : 0.22 mmol/g, CHN-analysis: 0.26 mmol/g). A ninhydrin assay gave a positive result.

*First amino acid coupling* The resin **31** was split into 18X150mg (18X0.036 mmol) portions in 10 mL PP vessels. Into each vessel was added 1.5 mL DMF solution containing a different 90% Fmoc-amino acid (0.13 mmol) and 10% N-acyl amino acid (0.0144 mmol). The vessels were then shaken and vortexed for half an hour followed by the addition of 1.5 mL DMF solution containing HBTU (0.144 mmol) and HOBt (0.144 mmol) into each vessel. After shaking the vessels for 1 min. the DIPEA (52 μL, 0.288 mmol) was added. The vessels were then vortexed for 2h until they were filtered and rinsed with DMF, MeOH and $CH_2Cl_2$ (3 times each). Ninhydrin assays of all 18 portions were positive. The Fmoc-amino acids were deprotected with two treatments with 1:4 piperidine in DMF (10 min. then 25 min.). Afterwards, the each portion of resin was washed with DMF, MeOH and $CH_2Cl_2$ (3 times each), dried under high-vacuum over 12h. From each was removed ~10 mg of resin for ES-MS analysis to

check the ratio of N-acyl amino acid signal to the Fmoc-amino acid signal. The contents of each vessels were then mixed thoroughly into one larger PP vessel that was shaken in $CH_2Cl_2$ for a few minutes to ensure homogeneity and then filtered and dried under high-vacuum for 24h. The average loading was only slightly changed due to low functionality of the starting resin.

3) General procedure for dipeptide library **33** (see Figure 16)

The resin **32** was split into 18X90mg (18X0.021mmol) portions in 10 mL PP vessels. A similar procedure to above was then followed using 1.0 mL DMF solution of 90% Fmoc-amino acid (0.079 mmol) and 10% N-acyl amino acid (0.0088mmol), 1.0 mL DMF solution of HBTU and HOBt (0.084 mmol, each), and then DIPEA (31 μL, 0.168 mmol). After deprotecting Fmoc groups 18 portions of resin were mixed and dried in the same way as above.

4) General procedure for polyamide library **34** (see Figure 16)

The resin **33** was split into 4X300mg (4X0.07mmol) portions in 10 mL PP vessels. To one portion was added a solution of 2,4,5-trichlorophenyl formate (85 mg, 0.37 mmol) in 2 mL DMF. The mixture was vortexed at RT for 3h, then washed with DMF, MeOH and $CH_2Cl_2$ (4 times each) and dried under high-vacuum over 24h. The sublibrary A of **34** was produced.

The other three portions were respectively capped with butyric acid, benzoic acid and t-butylacetic acid (0.30 mmol each) in 2 mL DMF using 2 mL DMF solution of HBTU and HOBt (0.30 mmol each) and then DIPEA (0.22 mL, 0.60 mmol). The workup was the same as usual. These processes gave three sublibraries B, C and D of polyamide library.

5) General procedure for polyamine library **35** (see Figure 16)

One sublibrary of **34** (~100 mg, ~0.024 mmol) was placed into a 5 mL silanized round bottom flask and swelled in dry THF (0.2 mL) under nitrogen. The diborane solution (1M in THF, 1.2 mL, 1.2 mmol) was added dropwise at rt over 1 min. The flask was then equipped with a condenser and the suspension was gently refluxed at 65°C for 4 days, followed by either Workup 1 or Workup 2 to afford the corresponding sublibrary of polyamine.

*Workup 1* Upon cooling to RT, the suspended resin was rapidly transferred into a PP filter vessel via a silanized pipette using dry THF to rinse out the flask and to wash the resin extensively. Then, dry THF (0.2 mL), anhydrous DIPEA (0.07 mL) and $EtCO_2H$ (0.14 mL) were added successively. After shaking the suspension the iodine was added (0.30g, 1.2 mmol in 0.59 mL THF) and the vessel was vortexed for 4h. The resin was then filtered and rinsed with THF, 1:3 $Et_3N$/DMF, MeOH and $CH_2Cl_2$ (3 times each) and dried under high-vacuum over 24h.

*Workup 2* The reaction was quenched with MeOH at 0°C. Following decantation of the reaction solution the resin was then washed with MeOH (1mLX3), THF (1mLX2) and piperidine (1mLX2). The amine-borane complex was then disproportionated by overnight treatment (16h) with 2mL piperidine at 65°C. The resin was transferred into a PP filter vessel, rinsed with DMF, MeOH and $CH_2Cl_2$ (4 times each), and dried under high-vacuum over 24h.

6) General procedure for "exo-peptide" library **36** (see Figure 16)

One sublibrary (~50 mg, ~ 0.012 mmol) of **35** was vortexed with triethylamine (21 µL, 0.145 mmol) in 0.5mL DMF for a few seconds followed by the addition of acetic anhydride (50 µL, 0.50 mmol). After vortexing for 2.5h, this $Ac_2O/Et_3N$ procedure was exactly repeated. Then, the resin was filtered and washed with DMF, $Et_3N$/DMF (1:3), DMF, MeOH and $CH_2Cl_2$ (4 times each), and dried under high-vacuum for 24h to provide the corresponding "exo-peptide" sublibrary.

7) Single bead cleavage and LC/MS analysis

*Single bead cleavage:* A small amount of resin (~1 mg) was spread onto a watch glass and placed on the stage of an inverted microscope. A 10 µL syringe was filled with 5 µL of 5% TFA/$CH_2Cl_2$ and then 2-5µL of air by pulling the plunger further upwards. Using the tip of the syringe needle a single bead was picked up from under microscope. The bead on the syringe tip was transferred into a microvial with the 5 µL 5% TFA/ $CH_2Cl_2$. The topped microvial was kept at RT for 2-3h followed by naturally evaporating the solvent into the air. The residue in the microvial was dissolved in 8µL methanol for both polyamide and "exo-peptide" libraries, and in 8µL $H_2O$/MeCN (1:1) containing 0.1% TFA for polyamine library.

*LC/MS analysis conditions*

**Polyamine library:** Method: PLYAMIN.M

Column: Zorbax SB-C8, 4.6X50 mm, 3.5 μm

Eluent: 15-85% MeCN (0.1 %TFA) in water (0.1% TFA) over 5 min. then maintained at 85% for another 7 min.

Flow rate: 0.7 mL/min.

Inj. volume: 2.5 μL

MS: Cap volt. 3200V; Frag. volt. 80V; neb. press. 40 psig; pas temp. 350°C; drying gas 10.0 L/min.

**Polyamide and "exo-peptide" libraries:**

Method: MEOHSEQ.M; 0.20 mL/min. MeOH pos ES for a sequence. Max pressure on the pump 15 psi.

Inj. volume: 2.5 μL

Regarding the observed peak ($MH^+$+24) that appeared in the polyamine **32** ES-MS spectrum in some cases, it was supposed that the peak was originally from either the $BH_3$-reduction or $I_2$-workup stage. The reduction of **31** to **32** was repeated under conditions as described in Ostresh (1998) J. Org. Chem. 63:8622-8623; Nefzi (2000) Tetrahedron 56:3319-3326; Nefzi (2000) Tetrahedron Lett. 41:5441-5446 ("Houghten's workup"); ($B(OH)_3$, 15 eq.; $B(OMe)_3$, 15 eq.; $BH_3$-THF, 45 eq.; 65°C/3d; workup: heating in piperidine at 65°C overnight) to give the expected clean polyamine **32** without any "+24" peak. Based on this result, a control experiment below was carried out. The resin-bound **31** was reduced using our $BH_3$/THF method (65°C/3d) under nitrogen and the Houghten's workup (in piperidine at 65°C overnight). The reaction afforded the polyamine **32** with higher purity and no "+24" peak was observed. This indicated that the $BH_3$-reduction worked well and the "+24" problem was possibly produced at the stage of $I_2$-workup. The problem was found in synthesis of another polyamine too. So it may be independent on the model compound **32**. On the other hand, "old" and "fresh" reagents of $BH_3$/THF were also compared in the same reaction. It showed only a little bit influence on "+24". The above-mentioned findings guided us to focus on improvement of $I_2$–workup procedure.

At first, two aqueous buffer systems (AcOH/AcONa, 0.2 M, pH 5.0; 2-$(HO_2C)C_6H_4CO_2K$/NaOH, 0.05 M, pH 5.0; 30% in THF for both) were tried in $I_2$-workup in consideration of this resin's hydrophilicity. The latter provided a relatively clean peak of $MH^+$+10. After treating the beads with ($HOCH_2CH_2OH$/DIPEA/THF. 4:2:7, vortexing at RT for 24h), the product peak ($MH^+$) was indeed major one, however, "+24" peak with 40% intensity still existed.

Next, the organic buffer system (DIPEA/AcOH/THF) was modified. The analysis of HRMS suggested that two structures (see **Figure 15C**) may have been responsible for the "+24". Structure I may have been related with acetic acid from buffer system of DIPEA/AcOH/THF. It was doubted that possible leftover acetic salt might be the problem. The intermediate I would be produced by the aid of TFA at the cleavage stage. However, a parallel experiment, where the resin was washed with or without $Et_3N$/DMF after $I_2$-workup, didn't show any significant difference. The reaction may have been "too anhydrous". A drop of water was added in the $I_2$-workup to break the structure I down in situ. However, this method still didn't solve the "+24" problem either. Using propionic acid as a substituent for acetic acid, results obtained indicated that propionic acid had slightly better performance; a small "+24" peak appeared in ES-MS again.

**Table 3 A part of experimental results regarding "+24" problem**

| Entry | Ratio (THF/$EtCO_2H$/DIPEA) | [$I_2$] g/ml buffer | Intensity (%) | | $MH^+/MH^+$+24 |
|---|---|---|---|---|---|
| | | | $H^+$ | $MH^+$+24 | |
| | 9:2:2 | 0.32 | 1 | 32 | 1:1.5 |
| | 9:2:1 | 0.36 | 00 | 18 | 5.6:1 |
| | 12:2:1 | 0.33 | 00 | 13 | 7.7:1 |
| | 15:2:1 | 0.32 | 00 | 5 | 20:1 |
| | 15:2:1 | 0.24 | 00 | 8 | 12.5:1 |

| | | | | | |
|---|---|---|---|---|---|
| | 7:2:1 | 0.39 | 00 | 62 | 1.6:1 |
| | 7:2:1 | 0.10 | 5 | 50 | N/A |

In order to break the possible borane-amine bonds shown in structure II (Figure 15C), the buffering ability of organic buffer and $I_2$-concentration in buffer may be key factors. This hypothesis is partly supported by the results listed in Table 1. When $EtCO_2H$/DIPEA was in the ratio of 1 to 1, "+24" peak was more intense than one of "$MH^+$" (entry 1). In this case the pH value of buffer was changed, which resulted in decreasing its buffering ability. Keeping $EtCO_2H$/DIPEA ratio of 2 to 1 was important. Varying length of vortexing during $I_2$-workup didn't help to solve the problem. On the other hand, "+24" peak was smaller and smaller as enhancement of THF proportion in buffer (entry 2-4). A remarkable effect of $I_2$-concentration was also observed (entry 4-7). The higher concentration was in favor of $MH^+$ peak. So far, the best ratio of $MH^+/(MH^++24)$ reached 20:1 by tuning [ $I_2$] and proportion of components of the organic buffer (entry 4). Although we didn't eliminate this "+24" peak it could be minimized under this workup conditions (THF:$EtCO_2H$:DIPEA=15:2:1, [ $I_2$]=0.30-0.40 g/ml buffer).

"Chlorotriarylmethane conjugated PEG-PS resin" is beneficial when using the combinatorial library to screen for biological, or other molecules, that can bind to the "exopeptides" or polyamines of the library.

Trioxa-1,13-tridecanediamine reacted with the resin in methylene chloride to produce $NH_2$-functionalized resin **31** (Figure 16) that was divided into 18 portions. Each portion was coupling with a different amino acid as a building block. Then, 18 resin-bound amino acids were mixed in dicholoromethane. This "split and mix" technique was applied again to construct a dipeptides library, followed by protecting the terminal free $NH_2$ groups with four different capping reagents. Thus, four sublibraries of **34** (Figure 16) were made. These sublibraries were reduced with the $BH_3/I_2$-method and acetylated with $Ac_2$/ $Et_3N$, respectively. The corresponding four polyamine sublibraries **35** (Figure 16) with about 1300 members and four "exo-

peptide" sublibraries **36** (Figure 16) possessing up to 10,368 rotamers were thus synthesized.

As illustrated in Figure 17, 18 amino acids were used as building blocks to make the initial resin-bound dipeptides library of **33** (Figure 16). Before initiating the library synthesis, two amino acids, Gln and Trp(Boc), were tested to determine if they could tolerate conditions used in the $BH_3/I_2$ method. This experiment revealed no problems for this transformation (for both Gln and the linker).

**Figure 18** illustrates the 18 acyl protected amino acids used as encoding compounds to identify combinatorial library members. They were obtained from one of three sources: (a) commercially available Ac-Ala-OH, Ac-Leu-OH, Ac-Met-OH, Ac-Phe-OH, Ac-Gln-OH, Ac-Gly-OH; (b) as prepared by method 1, above, n-Pr-C(=O)-D-Ala-OH, n-Pr-C(=O)-D-Met-OH, n-Pr-C(=O)-D-Leu-OH, n-Pr-C(=O)-D-Phe-OH, n-Pr-C(=O)-D-Nva-OH, n-Pr-C(=O)-D-Hfe-OH, n-Pr-C(=O)-D-Ser(tBu)-OH, n-Pr-C(=O)-D-Trp(Boc)-OH, n-Pr-$C_6H_{13}$-C(=O)-Nva-OH; (c) as prepared by method 2, above, Ac-Hfe-OH, Ac-Trp(Boc)-OH, Ac-Ser(tBu)-OH.

Four capping reagents, as shown in **Figure 19**, with larger differences in structure, were used at different stages.

A model exo-peptide compound **38** (as shown in **Figure 20**) with three differently bulky substituents (benzyl, methyl, t-butyl) was synthesized for checking its possible rotamers (up to 8).

*Partial termination encoding method*

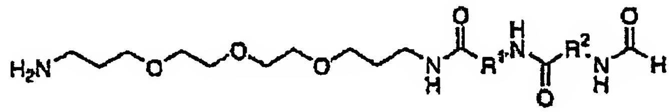
The $NH_2$-functionalized resin, as shown in Figure 21, reacted with a mixture of building block Fmoc-$AA_1$ and and $AA_1$ encoding reagent X-C(=O)- in the ratio of 9:1, followed by reacting with a second $AA_2$ in the same way, and capping with $R^3$ group to produce an encoded peptide. The building block and encoding reagent were supposed to have a similar reaction rate in peptide synthesis. The dipeptides and two encoding compounds attached to polymeric beads were estimated in a ratio of 8:1:1. Later, they were simultaneously reduced and acetylated during preparation of polyamine and "exo-peptide" libraries.

*Decoding process*

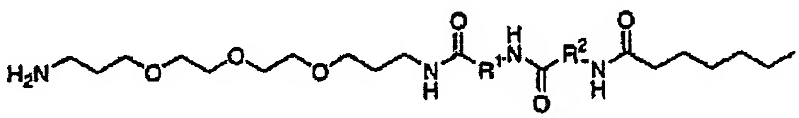
The nine pairs of amino acids (L-and D- forms) were used as building blocks to construct 12 sublibraries (four peptide sublibraries, four polyamine sublibraries and four exo-peptide sublibraries, corresponding to four capping groups, respectively). Because of the same mass of L- and D- amino acids, there are only 45 possible mass possibilities of combination of $R^1$ and $R^2$ for each sublibrary (Tables 4-6) [Note ot Dr. Hall – You indicated in your email of March 2002 to remove these tables. However, they will be needed to support this experiment and as such we have kept them in.]. Single beads were analyzed by ES-MS (for peptide and exo-peptide libraries) and LC-MS (for polyamine libraries) to determine which peak ($MH^+$) probably is a library member using tables 4-6. Then, sequence of two R groups and their stereochemistry (R- or L- forms) were identified according to mass of encoding compound I for $R^1$, and, based on mass of encoding compound II for $R^2$ (see Tables 7-9).

*Decoding process*

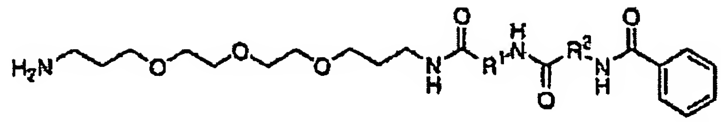
**Table 4 Member Masses of the Polvarnicle Library**



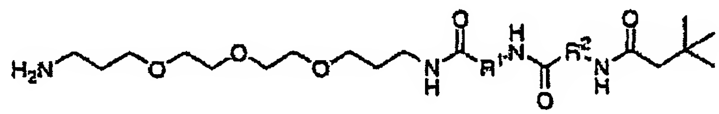
| R | Ala | Leu | Met | Phe | Ser(tBu) | Nva | Trp(Boc) | Gln | Hfe |
|---|---|---|---|---|---|---|---|---|---|
| Ala | 390.4 | 432.3 | 450.3 | 466.3 | 462.3 | 418.3 | 605.3 | 447.3 | 480.2 |
| Leu | | 474.3 | 492.3 | 508.3 | 504.4 | 460.3 | 647.4 | 489.3 | 522.3 |
| Met | | | 510.2 | 526.3 | 522.3 | 478.3 | 665.3 | 507.3 | 540.2 |
| Phe | | | | 542.3 | 538.3 | 494.3 | 681.4 | 523.3 | 556.3 |
| Ser(tBu) | | | | | 534.4 | 490.3 | 677.4 | 519.3 | 552.3 |
| Nva | | | | | | 446.3 | 633.4 | 475.3 | 508.3 |
| Trp(Boc) | | | | | | | 820.4 | 662.4 | 695.3 |
| Gln | | | | | | | | 504.3 | 537.3 |
| Hfe | | | | | | | | | 570.3 |



| R | Ala | Leu | Met | Phe | Ser(tBu) | Nva | Trp(Boc) | Gln | Hfe |
|---|---|---|---|---|---|---|---|---|---|
| Ala | 474.3 | 516.4 | 534.3 | 550.4 | 546.4 | 502.4 | 689.4 | 531.4 | 564.3 |
| Leu | | 558.4 | 576.4 | 592.4 | 588.4 | 544.4 | 731.5 | 573.4 | 606.4 |
| Met | | | 594.3 | 610.4 | 606.4 | 562.4 | 749.4 | 591.4 | 624.3 |
| Phe | | | | 626.4 | 622.4 | 578.4 | 765.5 | 607.4 | 640.4 |
| Ser(tBu) | | | | | 618.5 | 574.4 | 761.5 | 603.4 | 636.4 |
| Nva | | | | | | 530.4 | 717.5 | 559.4 | 592.4 |
| Trp(Boc) | | | | | | | 904.5 | 746.5 | 779.42 |
| Gln | | | | | | | | 588.4 | 621.4 |
| Hfe | | | | | | | | | 654.4 |

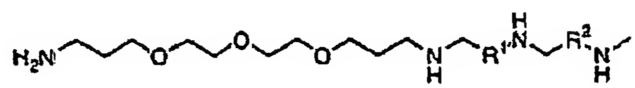


| R | Ala | Leu | Met | Phe | Ser(tBu) | Nva | Trp(Boc) | Gln | Hfe |
|---|---|---|---|---|---|---|---|---|---|
| Ala | 466.3 | 508.3 | 526.3 | 542.3 | 538.3 | 494.3 | 681.4 | 523.3 | 556.3 |
| Leu | | 550.4 | 568.3 | 584.4 | 580.4 | 536.4 | 723.4 | 565.4 | 598.4 |
| Met | | | 586.3 | 602.3 | 598.3 | 554.3 | 741.4 | 583.3 | 616.3 |
| Phe | | | | 618.3 | 614.4 | 570.3 | 757.4 | 599.3 | 632.3 |
| Ser(tBu) | | | | | 610.4 | 566.4 | 753.4 | 595.4 | 628.3 |
| Nva | | | | | | 522.3 | 709.4 | 551.3 | 584.3 |
| Trp(Boc) | | | | | | | 896.5 | 738.4 | 771.4 |
| Gln | | | | | | | | 580.3 | 613.3 |
| Hfe | | | | | | | | | 646.3 |

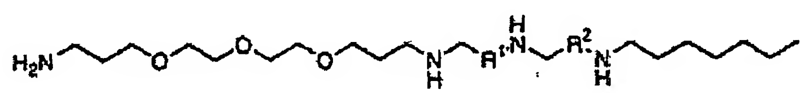


| R | Ala | Leu | Met | Phe | Ser(tBu) | Nva | Trp(Boc) | Gln | Hfe |
|---|---|---|---|---|---|---|---|---|---|
| Ala | 460.3 | 502.4 | 520.3 | 536.4 | 532.4 | 488.4 | 675.4 | 517.4 | 550.3 |
| Leu | | 544.4 | 562.4 | 578.4 | 574.4 | 530.4 | 717.5 | 559.4 | 592.4 |
| Met | | | 580.3 | 596.4 | 592.4 | 548.4 | 735.4 | 577.4 | 610.3 |
| Phe | | | | 612.4 | 608.4 | 564.4 | 751.5 | 593.4 | 626.4 |
| Ser(tBu) | | | | | 604.4 | 560.4 | 747.5 | 589.4 | 622.4 |
| Nva | | | | | | 516.4 | 703.5 | 545.4 | 578.4 |
| Trp(Boc) | | | | | | | 890.5 | 732.4 | 765.4 |
| Gln | | | | | | | | 574.4 | 607.3 |
| Hfe | | | | | | | | | 640.4 |

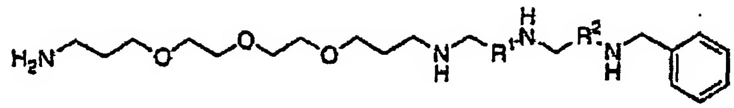
## Table 5 Member Masses of the Polyamine Library



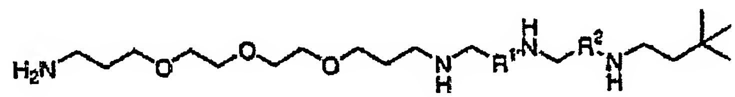
| R | Ala | Leu | Met | Phe | Ser(tBu) | Nva | Trp(Boc) | Gln | Hfe |
|---|---|---|---|---|---|---|---|---|---|
| Ala | 348.3 | 390.4 | 408.3 | 424.3 | 420.4 | 376.3 | 563.4 | 391.4 | 438.3 |
| Leu | | 432.4 | 450.4 | 466.4 | 462.4 | 418.4 | 605.5 | 433.4 | 480.4 |
| Met | | | 468.3 | 484.3 | 480.4 | 436.3 | 623.4 | 451.4 | 498.3 |
| Phe | | | | 500.4 | 496.4 | 452.4 | 639.4 | 467.4 | 514.3 |
| Ser(tBu) | | | | | 492.4 | 448.4 | 635.5 | 463.4 | 510.4 |
| Nva | | | | | | 404.4 | 591.4 | 419.4 | 466.3 |
| Trp(Boc) | | | | | | | 778.5 | 606.4 | 653.4 |
| Gln | | | | | | | | 434.4 | 481.3 |
| Hfe | | | | | | | | | 528.4 |



| R | Ala | Leu | Met | Phe | Ser(tBu) | Nva | Trp(Boc) | Gln | Hfe |
|---|---|---|---|---|---|---|---|---|---|
| Ala | 432.4 | 474.5 | 492.4 | 508.4 | 504.5 | 460.4 | 647.5 | 475.4 | 522.4 |
| Leu | | 516.5 | 534.5 | 550.5 | 546.5 | 502.5 | 689.5 | 517.5 | 564.5 |
| Met | | | 552.4 | 568.4 | 564.5 | 520.4 | 707.5 | 535.4 | 582.4 |
| Phe | | | | 584.5 | 580.5 | 536.5 | 723.5 | 551.5 | 598.4 |
| Ser(tBu) | | | | | 576.5 | 532.5 | 719.6 | 547.5 | 594.5 |
| Nva | | | | | | 488.5 | 675.5 | 503.5 | 550.4 |
| Trp(Boc) | | | | | | | 862.6 | 690.5 | 737.5 |
| Gln | | | | | | | | 518.5 | 565.4 |
| Hfe | | | | | | | | | 612.4 |

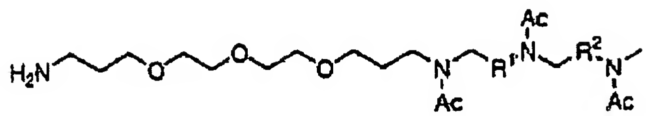


| R | Ala | Leu | Met | Phe | Ser(tBu) | Nva | Trp(Boc) | Gln | Hfe |
|---|---|---|---|---|---|---|---|---|---|
| Ala | 424.3 | 466.4 | 484.3 | 500.4 | 497.4 | 452.4 | 639.4 | 467.4 | 514.3 |
| Leu | | 508.4 | 526.4 | 542.4 | 538.4 | 494.4 | 681.5 | 509.4 | 556.4 |
| Met | | | 544.3 | 560.4 | 556.4 | 512.4 | 699.4 | 527.4 | 574.3 |
| Phe | | | | 576.4 | 572.4 | 528.4 | 715.5 | 543.4 | 590.4 |
| Ser(tBu) | | | | | 568.5 | 524.4 | 711.5 | 539.4 | 586.4 |
| Nva | | | | | | 480.4 | 667.5 | 495.4 | 540.4 |
| Trp(Boc) | | | | | | | 854.5 | 682.5 | 729.4 |
| Gln | | | | | | | | 510.4 | 557.4 |
| Hfe | | | | | | | | | 604.3 |

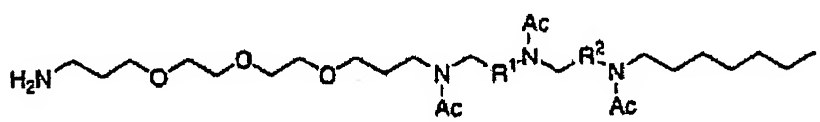


| R | Ala | Leu | Met | Phe | Ser(tBu) | Nva | Trp(Boc) | Gln | Hfe |
|---|---|---|---|---|---|---|---|---|---|
| Ala | 418.4 | 460.4 | 478.4 | 494.4 | 490.4 | 446.4 | 633.5 | 461.4 | 508.4 |
| Leu | | 502.5 | 520.4 | 536.5 | 532.5 | 488.5 | 675.5 | 503.5 | 550.5 |
| Met | | | 538.4 | 554.4 | 550.4 | 506.4 | 693.5 | 521.4 | 568.4 |
| Phe | | | | 570.5 | 566.5 | 522.5 | 709.5 | 537.5 | 584.5 |
| Ser(tBu) | | | | | 562.5 | 518.5 | 705.5 | 533.5 | 580.4 |
| Nva | | | | | | 474.5 | 661.5 | 489.5 | 536.4 |
| Trp(Boc) | | | | | | | 848.6 | 676.5 | 723.5 |
| Gln | | | | | | | | 504.5 | 551.4 |
| Hfe | | | | | | | | | 598.4 |

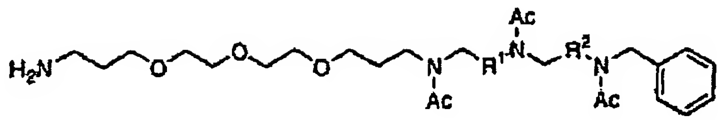
**Table 6 Member Masses of the "Exo-peptide" Library**



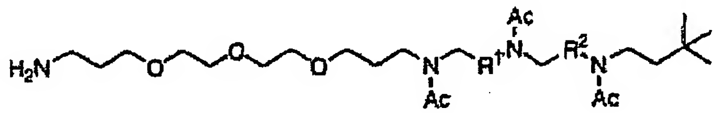
| R | Ala | Leu | Met | Phe | Ser(tBu) | Nva | Trp(Boc) | Gln | Hfe |
|---|---|---|---|---|---|---|---|---|---|
| Ala | 474.3 | 516.4 | 534.3 | 550.4 | 546.4 | 502.4 | 689.4 | 559.4 | 564.3 |
| Leu | | 558.4 | 576.4 | 592.4 | 588.4 | 544.4 | 731.5 | 601.4 | 606.4 |
| Met | | | 594.3 | 610.4 | 606.4 | 562.4 | 749.4 | 619.4 | 624.3 |
| Phe | | | | 626.4 | 622.4 | 578.4 | 765.5 | 635.4 | 640.4 |
| Ser(tBu) | | | | | 618.5 | 574.4 | 761.5 | 631.5 | 636.4 |
| Nva | | | | | | 530.4 | 717.5 | 587.4 | 592.4 |
| Trp(Boc) | | | | | | | 904.5 | 774.5 | 779.4 |
| Gln | | | | | | | | 644.4 | 649.4 |
| Hfe | | | | | | | | | 654.4 |



| R | Ala | Leu | Met | Phe | Ser(tBu) | Nva | Trp(Boc) | Gln | Hfe |
|---|---|---|---|---|---|---|---|---|---|
| Ala | 558.4 | 600.5 | 618.4 | 634.5 | 630.5 | 586.5 | 773.5 | 643.5 | 648.4 |
| Leu | | 642.5 | 660.5 | 676.5 | 672.5 | 628.5 | 815.6 | 685.5 | 690.5 |
| Met | | | 678.4 | 694.5 | 690.5 | 646.5 | 833.5 | 703.5 | 708.4 |
| Phe | | | | 710.5 | 706.5 | 662.5 | 849.6 | 719.5 | 724.5 |
| Ser(tBu) | | | | | 702.5 | 658.5 | 845.6 | 715.5 | 720.5 |
| Nva | | | | | | 614.5 | 801.6 | 671.5 | 676.5 |
| Trp(Boc) | | | | | | | 988.6 | 858.6 | 863.5 |
| Gln | | | | | | | | 728.5 | 733.5 |
| Hfe | | | | | | | | | 738.5 |



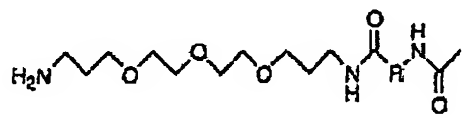
| R | Ala | Leu | Met | Phe | Ser(tBu) | Nva | Trp(Boc) | Gln | Hfe |
|---|---|---|---|---|---|---|---|---|---|
| Ala | 550.4 | 592.4 | 610.4 | 626.4 | 622.4 | 578.4 | 765.5 | 635.4 | 640.4 |
| Leu | | 634.5 | 652.4 | 668.5 | 664.5 | 620.5 | 807.5 | 677.5 | 682.5 |
| Met | | | 670.4 | 686.4 | 682.4 | 638.4 | 825.5 | 695.4 | 700.4 |
| Phe | | | | 702.4 | 698.5 | 654.4 | 841.5 | 711.5 | 716.4 |
| Ser(tBu) | | | | | 694.5 | 650.5 | 837.5 | 707.5 | 712.4 |
| Nva | | | | | | 606.4 | 793.5 | 663.5 | 668.4 |
| Trp(Boc) | | | | | | | 980.6 | 850.5 | 855.5 |
| Gln | | | | | | | | 720.5 | 725.4 |
| Hfe | | | | | | | | | 730.4 |



| R | Ala | Leu | Met | Phe | Ser(tBu) | Nva | Trp(Boc) | Gln | Hfe |
|---|---|---|---|---|---|---|---|---|---|
| Ala | 544.4 | 586.5 | 604.4 | 620.5 | 616.5 | 572.5 | 759.5 | 629.5 | 634.4 |
| Leu | | 628.5 | 646.5 | 662.5 | 658.5 | 614.5 | 801.6 | 671.5 | 676.5 |
| Met | | | 664.4 | 680.5 | 676.5 | 632.5 | 819.5 | 689.5 | 694.4 |
| Phe | | | | 696.5 | 692.5 | 648.5 | 835.5 | 705.5 | 710.4 |
| Ser(tBu) | | | | | 688.5 | 644.5 | 831.6 | 701.5 | 706.5 |
| Nva | | | | | | 600.5 | 787.5 | 657.5 | 662.4 |
| Trp(Boc) | | | | | | | 974.6 | 844.6 | 849.5 |
| Gln | | | | | | | | 714.5 | 719.5 |
| Hfe | | | | | | | | | 724.5 |

**Table 7 Encoding Compound Nlasses for the polyamide Library**

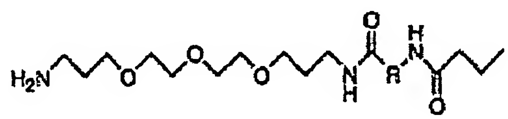
(1) If the first amino acid $R^1$ is L-form, mass of its encoding compound will be:



| R | Ala | Leu | Met | Phe | Ser(tBu) | Nva* | Trp(Boc) | Gln | Hfe |
|---|---|---|---|---|---|---|---|---|---|
| Mass | 333.2 | 375.3 | 393.2 | 409.2 | 405.3 | 431.3 | 508.3 | 390.2 | 423.3 |

*not the exactly same structure as shown above

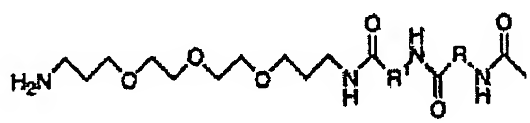
(2) If the first amino acid $R^1$ is D-form, mass of its encoding compound will be:



| R | Ala | Leu | Met | Phe | Ser(tBu) | Nva | Trp(Boc) | Gln* | Hfe |
|---|---|---|---|---|---|---|---|---|---|
| Mass | 361.2 | 403.3 | 421.2 | 437.2 | 433.3 | 389.3 | 576.3 | 319.2 | 451.3 |

*not the exactly same structure as shown above

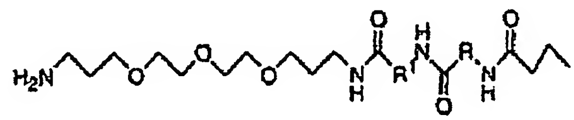
(3) If the second arruino acid $R^2$ is L-form, mass of its encoding compound will be:



| $R^1$(R) | Ala | Leu | Met | Phe | Ser(tBu) | Nva* | Trp(Boc) | Gln | Hfe |
|---|---|---|---|---|---|---|---|---|---|
| Ala | 404.2 | 446.3 | 464.2 | 480.3 | 476.3 | 502.3 | 619.3 | 461.2 | 494.3 |
| Leu | | 488.4 | 506.2 | 522.3 | 518.3 | 544.3 | 661.3 | 503.2 | 536.3 |
| Met | | | 524.2 | 540.3 | 536.3 | 562.3 | 679.3 | 521.2 | 554.3 |
| Phe | | | | 556.3 | 552.3 | 578.3 | 695.3 | 537.2 | 570.3 |
| Ser(tBu) | | | | | 548.4 | 574.4 | 691.4 | 533.3 | 566.4 |
| Nva | | | | | | 530.3 | 647.3 | 489.2 | 522.3 |
| Trp(Boc) | | | | | | | 834.4 | 676.3 | 709.4 |
| Gln | | | | | | | | 518.2 | 551.3 |
| Hfe | | | | | | | | | 584.4 |

*not the exactly same structure as shown above

(4) If the second amino acid $R^2$ is R-form, mass of its encoding compound will be:

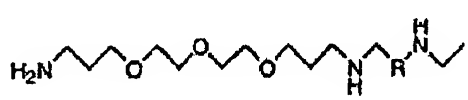


| $R^1$(R) | Ala | Leu | Met | Phe | Ser(tBu) | Nva | Trp(Boc) | Gly* | Hfe |
|---|---|---|---|---|---|---|---|---|---|
| Ala | 432.2 | 474.3 | 492.2 | 508.3 | 504.3 | 460.3 | 647.3 | 390.2 | 522.3 |
| Leu | | 516.4 | 534.2 | 550.4 | 546.3 | 502.3 | 689.3 | 432.2 | 564.3 |
| Met | | | 552.2 | 568.3 | 564.3 | 520.3 | 707.3 | 450.2 | 582.3 |
| Phe | | | | 584.3 | 580.3 | 536.3 | 723.3 | 466.2 | 598.3 |
| Ser(tBu) | | | | | 576.4 | 532.4 | 719.4 | 462.3 | 594.4 |
| Nva | | | | | | 488.3 | 675.3 | 418.2 | 550.3 |
| Trp(Boc) | | | | | | | 862.4 | 605.3 | 737.4 |
| Gln | 489.2 | 531.2 | 549.2 | 565.2 | 561.3 | 517.2 | 704.3 | 447.2 | 579.3 |
| Hfe | | | | | | | | | 612.4 |

*not the exactly same structure as shown above

**Table 8 Encoding Compound Masses for the polyamine Library**

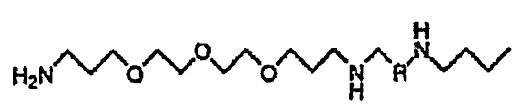
(1) If the first armino acid $R^1$ is L-form, mass of its encoding compound will be:



| R | Ala | Leu | Met | Phe | Ser(tBu) | Nva* | Trp(Boc) | Gln | Hfe |
|---|---|---|---|---|---|---|---|---|---|
| Mass | 305.2 | 347.3 | 365.2 | 381.2 | 377.3 | 403.3 | 520.3 | 348.3 | 395.3 |

*not the exactly same structure as shown above

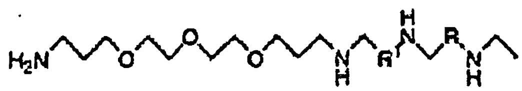
(2) If the first amino acid $R^1$ is D-form, mass of its encoding compound will be:



| R | Ala | Leu | Met | Phe | Ser(tBu) | Nva | Trp(Boc) | Gln* | Hfe |
|---|---|---|---|---|---|---|---|---|---|
| Mass | 333.2 | 375.3 | 393.2 | 409.2 | 405.3 | 361.3 | 548.3 | 277.3 | 423.3 |

*not the exactly same structure as shown above

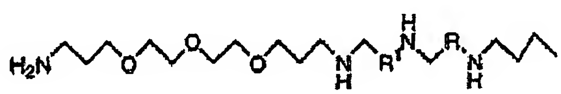
(3) If the second amino acid $R^2$ is L-form, mass of its encoding compound will be:



| $R^1$(R) | Ala | Leu | Met | Phe | Ser(tBu) | Nva* | Trp(Boc) | Gln | Hfe |
|---|---|---|---|---|---|---|---|---|---|
| Ala | 362.3 | 404.4 | 422.3 | 438.4 | 434.4 | 460.4 | 577.4 | 405.3 | 452.4 |
| Leu | | 446.5 | 464.3 | 480.4 | 476.4 | 502.4 | 619.4 | 447.3 | 494.4 |
| Met | | | 482.3 | 498.4 | 494.4 | 520.4 | 637.4 | 465.3 | 512.4 |
| Phe | | | | 514.4 | 510.4 | 536.4 | 653.4 | 481.3 | 528.4 |
| Ser(tBu) | | | | | 506.5 | 532.5 | 649.5 | 477.4 | 524.5 |
| Nva | | | | | | 488.4 | 605.4 | 433.3 | 480.4 |
| Trp(Boc) | | | | | | | 792.5 | 620.4 | 667.5 |
| Gln | | | | | | | | 448.3 | 495.4 |
| Hfe | | | | | | | | | 542.5 |

*not the exactly same structure as shown above

(4) If the second amino acid $R^2$ is R-form, mass of its encoding compound will be:

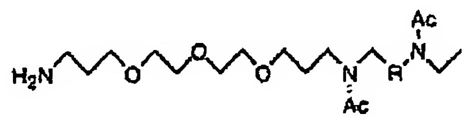


| $R^1$(R) | Ala | Leu | Met | Phe | Ser(tBu) | Nva | Trp(Boc) | Gly* | Hfe |
|---|---|---|---|---|---|---|---|---|---|
| Ala | 390.3 | 432.4 | 450.3 | 466.4 | 462.4 | 418.4 | 605.4 | 348.3 | 480.4 |
| Leu | | 474.5 | 492.3 | 508.5 | 504.4 | 460.4 | 647.4 | 390.3 | 522.4 |
| Met | | | 510.3 | 526.4 | 522.4 | 478.4 | 665.4 | 408.3 | 540.4 |
| Phe | | | | 542.4 | 538.4 | 494.4 | 681.4 | 424.3 | 556.4 |
| Ser(tBu) | | | | | 534.5 | 490.5 | 677.5 | 420.4 | 552.5 |
| Nva | | | | | | 446.4 | 633.4 | 376.3 | 508.4 |
| Trp(Boc) | | | | | | | 820.5 | 563.4 | 695.5 |
| Gln | 433.3 | 475.3 | 493.3 | 509.3 | 505.4 | 461.3 | 648.4 | 391.3 | 523.4 |
| Hfe | | | | | | | | | 570.5 |

*not the exactly same structure as shown above

**Table 9 Encoding Compound Masses for the "Exo-peptide" Library**

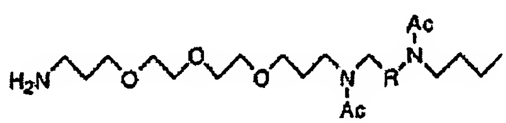
(1) If the first amino acid $R^1$ is L-form, mass of its encoding compound will be:



| R | Ala | Leu | Met | Phe | Ser(tBu) | Nva* | Trp(Boc) | Gln | Hfe |
|---|---|---|---|---|---|---|---|---|---|
| Mass | 389.3 | 431.3 | 449.3 | 465.3 | 461.4 | 487.4 | 604.4 | 474.3 | 479.4 |

*not the exactly same structure as shown above

(2) If the first amino acid $R^1$ is D-form, mass of its encoding compound will be:



| R | Ala | Leu | Met | Phe | Ser(tBu) | Nva | Trp(Boc) | Gln* | Hfe |
|---|---|---|---|---|---|---|---|---|---|
| Mass | 417.3 | 459.4 | 477.2 | 493.2 | 489.3 | 445.4 | 632.3 | 403.3 | 507.3 |

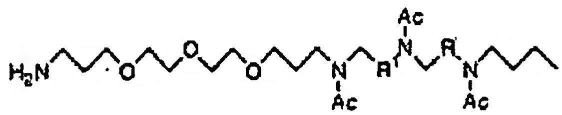
*not the exactly same structure as shown above

(3) If the second amino acid $R^2$ is L-form, mass of its encoding compound will be:

| $R^1$(R) | Ala | Leu | Met | Phe | Ser(tBu) | Nva* | Trp(Boc) | Gln | Hfe |
|---|---|---|---|---|---|---|---|---|---|
| Ala | 488.3 | 530.4 | 548.3 | 564.4 | 560.4 | 586.4 | 703.4 | 573.3 | 578.4 |
| Leu | | 572.5 | 590.3 | 606.4 | 602.4 | 628.4 | 745.4 | 615.3 | 620.4 |
| Met | | | 608.3 | 624.4 | 620.4 | 646.4 | 763.4 | 633.3 | 638.4 |
| Phe | | | | 640.4 | 636.4 | 662.4 | 779.4 | 649.3 | 654.4 |
| Ser(tBu) | | | | | 632.5 | 658.5 | 775.5 | 645.4 | 650.5 |
| Nva | | | | | | 614.4 | 731.4 | 601.3 | 606.4 |
| Trp(Boc) | | | | | | | 918.5 | 788.4 | 793.5 |
| Gln | | | | | | | | 658.4 | 663.4 |
| Hfe | | | | | | | | | 668.5 |

*not the exactly same structure as shown above

(4) If the second amino acid $R^3$ is R-form, mass of its encoding compound will be:



| $R^1(R)$ | Ala | Leu | Met | Phe | Ser(tBu) | Nva | Trp(Boc) | Gly* | Hfe |
|---|---|---|---|---|---|---|---|---|---|
| Ala | 516.3 | 558.4 | 576.3 | 592.4 | 588.4 | 544.4 | 731.4 | 516.3 | 607.4 |
| Leu | | 600.5 | 618.3 | 634.5 | 630.4 | 586.4 | 773.4 | 558.3 | 648.4 |
| Met | | | 636.3 | 652.4 | 648.4 | 604.4 | 791.4 | 576.3 | 666.4 |
| Phe | | | | 668.4 | 664.4 | 620.4 | 807.4 | 592.3 | 682.4 |
| Ser(tBu) | | | | | 660.5 | 616.5 | 803.5 | 588.4 | 678.5 |
| Nva | | | | | | 572.4 | 759.4 | 544.3 | 634.4 |
| Trp(Boc) | | | | | | | 946.5 | 731.4 | 821.5 |
| Gln | 559.3 | 601.4 | 619.3 | 635.4 | 631.4 | 587.4 | 774.4 | 601.3 | 691.4 |
| Hfe | | | | | | | | | 696.5 |

*not the exactly same structure as shown above

*Decoding results*

The peptide library was easily decoded by single bead's ES-MS in average 90%. In the partially decoded cases, $MH^+$ peak could be found but molecular peaks of one or two encoding compounds were too weak to be distinguished, thus, sequence and stereochemistry of those members could not be completely determined. Two beads were unreadable.

**Table 10 Peptide library**

| $R^3$ | No. of beads isolated | No. of beads completely decoded | No. of beads partially decoded | No. of unreadable beads | Decoded (%) |
|---|---|---|---|---|---|
| A | 10 | 7 | 2 | 1 | 80 |
| B | 10 | 9 | 0 | 1 | 90 |
| C | 10 | 8 | 2 | 0 | 90 |
| D | 10 | 10 | 0 | 0 | 100 |

The polyamine library was also decoded using the LC-MS technique. Under some circumstances, no peak was observed, and curve was fat in LC-MS, which

resulted in undecoding. Only a portion of the beads were decoded due the same causes as note above for the peptide libraries.

**Table 11 Polyamine library**

| $R^3$ | No. of beads isolated | No. of beads completely decoded | No. of beads partially decoded | No. of unreadable beads | Decoded (%) |
|---|---|---|---|---|---|
| A | 10 | 3 | 3 | 4 | 45 |
| B | 10 | 7 | 1 | 2 | 75 |
| C | 10 | 6 | 2 | 2 | 70 |
| D | 9 | 7 | 0 | 2 | 77 |

Fifteen beads of model polyamine 7 were analyzed in the same manner and results were obtained as follows:

| Number of beads | Intensity of $MH^+$ peak |
|---|---|
| 10 | strong |
| 2 | weaker |
| 3 | weak |

This data suggests that the beads' loading was not uniform and a part of them possessed lower loading. The resin has under gone a series of chemical transformations, and, it is conceivable that each bead did not react in exactly the same way in the solid phase reactions; this could be due to a variety of reasons. For example, differences in their loading was accumulated step by step. Alternatively, the presence of encoding compounds also decreased library members' loading on the resin, compared to model polyamine 7. The reaction rates in peptide synthesis were not the same for building block amino acids and their corresponding encoding reagents. The may be because loadings of library members or encoding compounds were smaller than calculated values. Another consideration is the sensitivity LC-MS readings. Thus, beads with low loading values were difficult to decode. Alternatively, the cause may be in the reduction step for sublibrary A.

**Table 12 Exo-Peptide library**

| $R^3$ | No. of beads isolated | No. of beads completely decoded | No. of beads partially decoded | No. of unreadable beads | Decoded (%) |
|---|---|---|---|---|---|
| A | 20 | 7 | 1 | 12 | 32 |
| B | 20 | 7 | 2 | 11 | 40 |
| C | 20 | 10 | 4 | 6 | 60 |
| D | 25 | 16 | 4 | 5 | 70 |

The "exo-peptide" library yielded the poorest decoding results. May beads did not afford significant information and PEG moieties were predominant in their MS.

**EXAMPLE 4- Derivatization of polyamine libraries to produce libraries for the recognition of oligosaccharides via the fromation of boronate esters.**

Boronation of TentaGel supported triamine library:

The resin-bound triamine library (237 mg, 0.0332 mmol at 0.14 mmol/g)is weighed inside a 25 mL round bottom flask and swelled with 6.6 mL ofanhydrous THF. As the suspension is being gently swirlled, 1,2,2,5,5-pentamethylpiperidine (PMP) (0.18 mL, 0.996 mmol) is added followed bythe 2-bromomethylboronic ester (0.18 mL, 0.996 mmol). A condenser isthen placed on the flask and is placed in a 65oC oil bath for 2 days. Notethat no stirring of the suspension with a stir bar is done as it will damagethe resin beads. The suspension is then cooled and transferred via a pipette to a polypropylene filter vessel where it is rinsed with THF (4x). The resin is then rinsed with water (2x; 1 minute and 30 minutes),THF(4x), methanol (4x) and then dichloromethane (5x). It is then dried under high vacuum for 16 to 24 hours.

A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, other embodiments are within the scope of the following claims.

[1] Cohen, S.S. A Guide to the Polyamines, Oxford University Press: Oxford, New York. **1999.**

[2] For recent reviews on the synthesis and biological activity of polyamine analogues, see: (a) Casero, R.A., Jr.; Woster, P.M. *J. Med. Chem.* **2001**, *44*, 1-26. (b) Karigiannis, G.; Papaioannou, D. *Eur. J. Org. Chem.* **2000**, 1841-1863. (c) Kuksa, V.; Buchan, R.; Lin, P.K.J. *Synthesis* **2000**, 1189-1207.

[3] For examples, see: DNA: (a) Deng, H.; Bloomfield, V.A.; Benevides, S.M.; Thomas, G.J. Jr, *Nucl. Acid Res.* **2001**, *17*, 3379-3385. RNA: (b) Quigley, G.J.; Teeter, M.M.; Rich, A. *Proc. Natl. Acad. Sci. USA* **1978**, *75*, 64-68. Inositol-tris(phosphates): (c) Mernissi-Arifi, K.; Zenkouar, M.; Schlewer, G.; Spiess, B. *J. Chem. Soc., Faraday Trans.* **1996**, *92*, 3101-3107.

[4] Shilo, M. in *Microbial Toxins*, Vol. VII (Eds.: Kadis, S.; Ciegler, A.; Ajl, S.J.), Academic Press, New York, 1971, pp. 67-103.

[5] (a) Tabet, M.; Labroo, V.; Sheppard, P.; Sasaki, T. *J. Am. Chem. Soc.* **1993**, *115*, 3866-3868. (b) Peczuh, M.W.; Hamilton, A.D.; Sánchez-Quesada, J.; de Mendoza, J.; Haack, T.; Giralt, E. *J. Am. Chem. Soc.* **1997**, *119*, 9327-9328.

[6] For one example on the calcium-binding protein parvalbumin,see: (a) Sudhakar, K.; Erecinska, M.; Vanderkooi, J.M. *Eur. J. Biochem.* **1995**, *230*, 498-502. For a review on the interactions of polyamines with ion channel proteins, see: (b) Williams, K. *Biochem. J.* **1997**, *325*, 289-297.

[7]. For seminal examples on the "inverse" approach involving selected cyclic or acyclic polyamines with several linear diacids, see: (a) Hosseini, M.W.; Lehn, J.-M. *Helv. Chim. Acta.* **1986**, *69*, 587-593. (b) Hossain, M.A.; Schneider, H.-J. *Chem. Eur. J.* **1999**, *5*, 1284-1290.

[8] The competing effect of water has been known as a notorious frustration in the design of synthetic receptors based on hydrogen bonds. For reviews on receptors for anion recognition, see: (a) Schmidtchen, F.P.; Berger, M. *Chem. Rev.* **1997**, *97*, 1609-1646. (b) Beer, P.D.; Gale, P.A. *Angew. Chem. Int. Ed.* **2001**, *40*, 486-516.

[9] For reviews on "one bead-one molecule" libraries made by split-pool synthesis, see: (a) Lam, K.M.; Lebl, M.; Krchnák, V. *Chem. Rev.* **1997**, *97*, 411-448. (b) Still, W.C. *Acc. Chem. Res.* **1996**, *29*, 155-163.

[10]. For other examples on dye screening, see: (a) Lam, K.S.; Zhao, Z.-G.; Wade, S.; Krchnák, V.; Lebl, M. *Drug. Dev. Res.* 1994, *33*, 157-160. (b) Wennemers, H.; Still, W.C. *Tetrahedron Lett.* 1994, *35*, 6413-6416.

[11] van Boeckel, C.A.A.; Petitou, M. *Angew. Chem. Int. Ed. Engl.* 1993, *32*, 1671-1690.

[12] Ostresh, J.M.; Husar, G.M.; Blondelle, S.E.; Dörner, B.; Weber, P.A.; Houghten, R.A. *Proc. Natl. Acad. Sci. USA* 1994, *91*, 11138-11142.

[13] (a) Manku, S.; Laplante, C.; Kopac, D.; Chan, T.; Hall, D.G. *J. Org. Chem.* 2001, *66*, 874-885. (b) Wang, F.; Manku, S.; Hall, D.G. *Org. Lett.* 2000, *2*, 1581-1583.

[14] See details in Supporting Information section. The final resin loading of the polyamine libraries is relatively high (ca. 0.7 $mmolg^{-1}$). Due to the hydrophobic nature of the polymer matrix it is possible that polyamine protonation is uniform mainly at the surface. This should not affect binding selectivity. Similarly, the hindered tritylamine anchor is not expected to interfere.

[15] Youngquist, R.S.; Fuentes, G.R.; Lacey, M.P.; Keough, T. *J. Am. Chem. Soc.* 1995, *117*, 3900.

[16] *N*-Butyryl derivatives of D-α-amino acids were employed in order to distinguish them from the L-enantiomer, and also to distinguish 4Acc from 2Acc by mass spectrometry.

[17] Bergeron, R.J.; Weimar, W.R.; Wu, Q.; Feng, Y.; McManis, J.S. *J. Med. Chem.* 1996, *39*, 5257-5266.

[18] A notable exception is the lower occurrence of $8Aoc^R$ at lower pH. Spermidine, which distant nitrogens are spaced by 8 atoms, might be a more effective competitor of this spacer under these conditions.

[19] Fielding, L. *Tetrahedron* 2000, *56*, 6151-6170.

[20] Performed in 20% $D_2O$ in DMSO-$d_6$ using standard methods described in reference 19 (see Supporting Information for more details). It should be noted that different binding stoichiometries may be possible on solid support due to the high local concentration of triamines within the beads.

[21] Geometrical optimization was performed using the SYBYL force field on MacSpartan Plus version 1.1.9 (Wavefunction Inc.).

[22] Performed in 20% $D_2O$ in DMSO-$d_6$. $K_a$ values were determined using the 1:1 complexation model program developed by C.A. Hunter (see Supporting Information for more details).

[23] See graphics in Abstract. Molecular docking of dye 2 with the protonated EtNH-2Acc$^R$-6Ahx$^R$-Et triamine, in a highly extended form, showed a high level of overlay between the *meta* sulfonates and the 2Acc$^R$ unit, with the 6-carbon interammonium spacer of 6Ahx$^R$ reaching the leftover sulfonate.

**WHAT IS CLAIMED IS:**

1. A method for the synthesis of a protected benzophenone-substituted resin comprising the following steps:

(a) providing a haloalkyl-terminated resin having a formula Resin-$(CH_2)_y$ X, wherein y is an integer between 1 and 20 and X is a halogen;

(b) providing a halo-magnesium-conjugated benzophenone derivative having a formula X-Mg-$(C_6H_4)$-$C(OR)_2$-Ph, wherein R is an alkyl substituent, X is a halogen, and $C_6H_4$ is a disubstituted benzene derivative; and,

(c) reacting the haloalkyl-terminated resin of step (a) with the halo-magnesium-conjugated benzophenone derivative of step (b) under conditions comprising a CuBr salt, a CuI salt or equivalent, and an anhydrous solvent, thereby producing a protected benzophenone-substituted resin having a formula Resin-$(CH_2)_y$ -$(C_6H_4)$-$C(OR)_2$-Ph, wherein y is an integer between 2 and about 25.

2. The method of claim 1, wherein the haloalkyl-terminated resin comprises a polystyrene (PS) end-functionalized with a haloalkyl-terminated hydrophilic polyethylene glycol polymer having a formula PS-$(CH_2)_m$ -$(OCH_2CH_2)_n$ -O$(CH_2)_y$-X, wherein X is a halogen, n is an integer between 1 and about 100, m is 1 to about 20, and y is an integer between 1 and about 25.

3. The method of claim 2, wherein the haloalkyl-terminated resin comprises Tentagel™.

4. The method of claim 2, wherein the hydrophilic polyethylene glycol polymer is branched.

5. The method of claim 4, wherein the haloalkyl-terminated resin comprises Argogel™.

6. The method of claim 2, wherein the haloalkyl-terminated resin comprises a polyoxyethylene-polyoxypropylene (POEPOP) or a polyoxyethylene-polyoxetane (SPOCC).

7. The method of claim 2, wherein the polystyrene resin comprises about 1% to about 2% crosslinked divinylbenzene.

8. The method of claim 1, wherein the haloalkyl-terminated resin comprise a beaded diameter between about 10 microns and about 500 microns.

9. The method of claim 1, wherein the halo-magnesium-conjugated benzophenone comprises a magnesium bromide-conjugated protected benzophenone.

10. The method of claim 1, wherein the halo-magnesium-conjugated benzophenone comprises a protected 4-X-Mg-benzophenone, wherein X is Cl, Br or I.

11. The method of claim 1, wherein the reactions conditions of step (c) comprise stirring for about one to four days.

12. The method of claim 1, wherein the reactions conditions of step (c) further comprise quenching with a neutral or mildly acidic solution.

13. The method of claim 12, wherein the solution comprises an ammonium salt, or equivalent.

14. A protected benzophenone-substituted resin produced by a method comprising the following steps:

(a) providing a haloalkyl-terminated resin having a formula Resin-$(CH_2)_y$X, wherein y is an integer between 1 and 20 and X is a halogen;

(b) providing a halo-magnesium-conjugated benzophenone derivative having a formula X-Mg-$(C_6H_4)$-C$(OR)_2$-Ph, wherein R is an alkyl substituent, the ketone group is protected as an acetal group, X is a halogen; and,

(c) reacting the haloalkyl-terminated resin of step (a) with the halo-magnesium-conjugated benzophenone of step (b) under conditions comprising a CuBr salt, or equivalent, and an anhydrous solvent, thereby producing a benzophenone-substituted resin having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-C$(OR)_2$-Ph, wherein y is an integer between 1 and about 25.

15. A protected benzophenone-substituted resin having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-C$(OR)_2$-Ph, wherein y is an integer between 1 and about 25.

16. A method for the synthesis of a deprotected benzophenone having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-CO-Ph comprising the following steps:

(a) providing a haloalkyl-terminated resin having a formula resin-$(CH_2)_y$X, wherein y is an integer between 1 and about 25 and X is a halogen;

(b) providing a halo-magnesium-conjugated benzophenone derivative having a formula X-Mg-$(C_6H_4)$-C$(OR)_2$-Ph, wherein R is an alkyl substituent, the ketone group is protected as an acetal group, X is a halogen; and,

(c) reacting the haloalkyl-terminated resin of step (a) with the halo-magnesium-conjugated benzophenone of step (b) under conditions comprising a CuBr salt, or equivalent, and an anhydrous solvent, thereby producing a benzophenone-substituted resin having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-$C(OR)_2$-Ph, wherein y is an integer between 1 and about 25;

(d) reacting the benzophenone-substituted resin of step (c) with an aqueous solution of $HClO_4$, or equivalent acid, and $CH_2Cl_2$, or equivalent; and

(e) rinsing and drying the resin, thereby producing an free benzophenone having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-CO-Ph, wherein y is an integer between 1 and about 25.

17. The method of claim 16, wherein the reaction of step (d) comprises mixing the beads at about room temperature for between about 2 to about 24 hours.

18. The method of claim 16, wherein in step (e) the beads are rinsed with $H_2O$/THF (1:1), or equivalent, DMF/$Et_3N$ (1:3), or equivalent, MeOH, or equivalent, and $CH_2Cl_2$, or equivalent.

19. The method of claim 16, wherein in step (e) the beads are rinsed several times.

20. A method for the synthesis of a deprotected benzophenone having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-CO-Ph comprising the following steps:

(a) providing a protected benzophenone-substituted resin having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-$C(OR)_2$-Ph, wherein y is an integer between 1 and about 25;

(b) reacting the benzophenone-substituted resin of step (c) with an aqueous solution of $HClO_4$, or equivalent acid, and $CH_2Cl_2$, or equivalent; and

(c) rinsing and drying the resin, thereby producing a deprotected benzophenone having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-CO-Ph, wherein y is an integer between 1 and about 25.

21. A deprotected benzophenone having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-CO-Ph made by a method comprising the following steps:

(a) providing a protected benzophenone-substituted resin having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-$C(OR)_2$-Ph, wherein y is an integer between 1 and about 25;

(b) reacting the benzophenone-substituted resin of step (a) with an aqueous solution of $HClO_4$, or equivalent acid, and $CH_2Cl_2$, or equivalent; and

(c) rinsing and drying the resin, thereby producing a deprotected benzophenone having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-CO-Ph.

22. A deprotected benzophenone having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-CO-Ph, wherein y is an integer between 1 and about 25.

23. A method for the synthesis of a hydroxy-triarylmethane-conjugated resin comprising the following steps:

(a) providing a haloalkylterminated resin having a formula resin-$(CH_2)_y$X, wherein y is an integer between 1 and about 25 and X is a halogen;

(b) providing a halo-magnesium-conjugated benzophenone having a formula X-Mg-$(C_6H_4)$-$C(OR)_2$-Ph, wherein R is an alkyl substituent, the ketone group is protected as an acetal group, X is a halogen;

(c) reacting the haloalkyl-terminated resin of step (a) with the halo-magnesium-conjugated benzophenone of step (b) under conditions comprising a CuBr salt, or equivalent, and an anhydrous solvent, thereby producing a benzophenone-substituted resin having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-$C(OR)_2$-Ph, wherein y is an integer between 1 and about 25;

(d) reacting the benzophenone-substituted resin of step (c) with an aqueous solution of $HClO_4$, or equivalent acid, and $CH_2Cl_2$, or equivalent;

(e) rinsing and drying the resin, thereby producing a deprotected benzophenone having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-CO-Ph, wherein y is an integer between 1 and about 25;

(f) mixing a suspension of the resin produced in step (e) with a solution comprising an arylmagnesium halide, or equivalent; and,

(g) adding an acidic solution, thereby producing a hydroxy-triarylmethane-conjugated resin having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-C(OH)(Ar)-Ph, wherein y is an integer between 1 and about 25.

24. The method of claim 23, further comprising washing and drying the hydroxy-triarylmethane-conjugated resin produced in step (g).

25. The method of claim 24, wherein washing conditions comprise washing with a solution comprising $H_2O$, an aqueous solution of

$NaHCO_3$, or equivalent, $H_2O$/THF (1:1), or equivalent, MeOH and $CH_2Cl_2$, or equivalent.

26. The method of claim 24, wherein drying conditions comprise high vacuum for greater than 12 hours in a dessicator or a glass vessel containing a drying agent.

27. The method of claim 23, wherein in step (f) the arylmagnesium halide comprises a phenylmagnesium halide, or equivalent.

28. The method of claim 23, wherein in step (f) the arylmagnesium halide is a functionalized biarylmagnesium halide having a formula XMg-$C_6H_4$-Y-$C_6H_4$-X, where Y is a selectively cleavable functionality, and X is a halide.

29. The method of claim 28, wherein the selectively cleavable functionality is selected from the group consisting of $CH_2OCH_2$, $CH_2SCH_2$, S, Se, Si, $CH_2SeCH_2$, $CH_2Si(Me)_2CH_2$, $SeCH_2$, $SCH_2$, $Si(Me)_2CH_2$, $CH_2Se$, $CH_2Si(Me)_2$, $CH_2S$ and equivalents.

30. The method of claim 28, wherein the hydroxy-triarylmethane-conjugated resin has a formula Resin-$(CH_2)_y$-$C_6H_4$-C(OH)($C_6H_4$-Y-$C_6H_4$-X)-Ph, wherein y is an integer between 1 and about 25.

31. The method of claim 23, wherein in step (f) the arylmagnesium halide is added dropwise under conditions comprising about room temperature.

32. A method for making a hydroxy-triarylmethane-conjugated resin comprising the following steps:

(a) providing a deprotected benzophenone having a formula Resin-$(CH_2)_y$-($C_6H_4$)-CO-Ph;

(b) mixing a suspension of the resin of step (a) with a solution comprising an arylmagnesium halide, or equivalent; and,

(c) adding an acidic solution, thereby producing a hydroxy-triarylmethane-conjugated resin having a formula Resin-$(CH_2)_y$-($C_6H_4$)-C(OH)(Ar)-Ph, wherein y is an integer between 1 and about 25.

33. A hydroxy-triarylmethane-conjugated resin made by a method comprising the following steps:

(a) providing a deprotected benzophenone having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-CO-Ph;

(b) mixing a suspension of the resin of step (a) with a solution comprising an arylmagnesium halide, or equivalent; and,

(c) adding an acidic solution, thereby producing a triarylmethane - conjugated resin having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-C(OH)(Ar)-Ph, wherein y is an integer between 1 and about 25.

34. A hydroxy-triarylmethane-conjugated resin having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-C(OH)(Ar)-Ph, wherein y is an integer between 1 and about 25.

35. A method for the synthesis of a chlorotriarylmethane-conjugated resin comprising the following steps:

(a) providing a haloalkyl-terminated resin having a formula resin-$(CH_2)_y$X, wherein y is an integer between 1 and 20 and X is a halogen;

(b) providing a halo-magnesium-conjugated benzophenone having a formula X-Mg-$(C_6H_4)$-$C(OR)_2$-Ph, wherein R is an alkyl substituent, the ketone group is protected as an acetal group, X is a halogen; and,

(c) reacting the haloalkyl-terminated resin of step (a) with the halo-magnesium-conjugated benzophenone of step (b) under conditions comprising a CuBr salt, or equivalent, and an anhydrous solvent, thereby producing a benzophenone-substituted resin having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-$C(OR)_2$-Ph, wherein y is an integer between 1 and about 25;

(d) reacting the benzophenone-substituted resin of step (c) with an aqueous solution of a strong acid and $CH_2Cl_2$, or equivalent;

(e) rinsing and drying the resin, thereby producing a deprotected benzophenone having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-CO-Ph, wherein y is an integer between 1 and about 25;

(f) mixing a suspension of the resin produced in step (e) with a solution comprising an arylmagnesium halide, or equivalent;

(g) adding an acidic solution, thereby producing a hydroxy-triarylmethane -conjugated resin having a formula Resin-$(CH_2)_y$ -$(C_6H_4)$-C(OH)(Ar)-Ph, wherein y is an integer between 1 and about 25;

(h) swelling the hydroxy-triarylmethane-conjugated resin of step (g) in dry $CH_2Cl_2$, or equivalent; and,

(i) mixing the swelled hydroxy-triarylmethane-conjugated of step (h) with a solution comprising a $SOCl_2$ or an acetyl chloride or equivalent, thereby producing a chlorotriarylmethane-conjugated resin having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-C(Cl)(Ar)-Ph, wherein y is an integer between 1 and 25.

36. The method of claim 35, further comprising drying the chlorotriarylmethane resin of step (i) under vacuum.

37. A method for the synthesis of a chlorotriarylmethane resin having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-C(Cl)(Ar)-Ph, wherein y is an integer between 1 and about 25, comprising the following steps:

(a) providing a hydroxy-triarylmethane-conjugated resin having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-C(OH)(Ar)-Ph, wherein y is an integer between 1 and about 25;

(b) swelling the hydroxy-triarylmethane resin in dry $CH_2Cl_2$, or equivalent; and,

(c) mixing the swelled resin of step (b) with a solution comprising a $SOCl_2$, or equivalent, thereby producing a chlorotriarylmethane resin having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-C(Cl)(Ar)-Ph, wherein y is an integer between 1 and about 25.

38. A chlorotriarylmethane resin having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-C(Cl)(Ar)-Ph made by a method comprising the following steps:

(a) providing a hydroxy-triarylmethane-conjugated resin having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-C(OH)(Ar)-Ph, wherein y is an integer between 1 and about 25;

(b) swelling the hydroxy-triarylmethane resin in dry $CH_2Cl_2$, or equivalent; and,

(c) mixing the swelled resin of step (b) with a solution comprising a $SOCl_2$, an acetyl chloride, or an equivalent, thereby producing a chlorotriarylmethane resin having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-C(Cl)(Ar)-Ph, wherein y is an integer between 1 and about 25.

39. A chlorotriarylmethane resin having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-C(Cl)(Ar)-Ph, wherein y is an integer between 1 and about 25.

40. The chlorotriarylmethane resin of claim 39, wherein the triarylmethane resin is a trityl resin.

41. A method of making a spacer-conjugated triarylmethane resin, wherein the spacer comprises the general formula -W-R-ZH, where W and Z are selected from the group consisting of O, S, and NH, and R is an alkyl or an oxyalkyl spacer or equivalent or oligomeric version thereof, comprising the following steps:

(a) providing a chlorotriarylmethane resin having a formula Resin-$(CH_2)_y$-$(C_6H_4)$-C(Cl)(Ar)(Ph), wherein y is an integer between 1 and about 25; and,

(b) reacting the resin of step (a) with HW-R-ZH, where W and Z are selected from the group consisting of O, S, and NH, and R is an alkyl or an oxyalkyl spacer or equivalent or oligomeric version thereof, and $CH_2Cl_2$, or equivalent, thereby making the spacer-conjugated triarylmethane resin.

42. The method of claim 41, wherein -W-R-ZH is -W-$(CH_2)_k$-[O-$(CH_2)_m]_n$-O-$(CH_2)_k$-ZH, wherein m and n are integers between about 0 and about 25, and k is an integer between about 2 and about 25.

43. The method of claim 42, wherein HW-$(CH_2)_k$-[O-$(CH_2)_m]_n$-O-$(CH_2)_k$-ZH is HW-$(CH_2)_2$-[O-$(CH_2)_2]_n$-O-$(CH_2)_2$-ZH.

44. The method of claim 42, wherein -W-R-ZH is - NH-$(CH_2)_k$-[O-$(CH_2)_m]_n$-O-$(CH_2)_k$- $NH_2$.

45. The method of claim 43, wherin – NH-$(CH_2)_k$ –[O-$(CH_2)_m]_n$-O-$(CH_2)_k$-$NH_2$ is –NH-$(CH_2)_3$-[O-$(CH_2)_2]_2$-O-$(CH_2)_3$-$NH_2$.

46. The method of claim 41, wherein the spacer-conjugated triarylmethane resin is Resin-$(CH_2)_k$ -[O-$(CH_2)_m]_n$-O-$(CH_2)_k$-ZH, wherein m and n are integers between about 0 and about 25, and k is an integer between about 2 and about 25.

47. A method of making a triarylmethane resin-conjugated peptide library comprising the following steps:

(a) providing a spacer-conjugated triarylmethane resin, wherein the spacer comprises the general formula -W-R-ZH, where W and Z are selected from

the group consisting of O, S, and NH, and R is an alkyl or an oxyalkyl spacer or equivalent or oligomeric version thereof;

(b) providing a plurality of 9-fluorenylmethoxycarbonyl (Fmoc) protected amino acid samples, wherein an amino acid comprises the general formula $HO_2C$-R'-NHFmoc;

(c) mixing each amino acid sample of step (b) with a spacer-conjugated triarylmethane resin of step (a) by a 9-fluorenylmethoxycarbonyl (Fmoc) procedure with a carbodiimide reagent, a hydroxybenzotriazole and a base or a coupling reagent and a base;

(d) releasing the terminal Fmoc group by stirring the resin of step (c) in an about 20 to 30% solution of piperidine, or equivalent, in a solvent selected from the group consisting of a dichloromethane, a DMF, a NMP, a THF and an equivalent, thereby making a deprotected resin;

(e) pooling the contents of all individual vessels, mixing, and redividing into equal amounts in the same number of samples as in step (b); and

(f) repeat steps (c) to (e) until the peptide has the desired length, thereby making the resin-conjugated peptide library.

48. The method of claim 47, wherein the spacer-conjugated triarylmethane resin is Resin-$(CH_2)_k$-$[O-(CH_2)_m]_n$-O-$(CH_2)_k$-ZH, wherein m and n are integers between about 0 and about 25, and k is an integer between about 2 and about 25.

49. The method of claim 47, wherein each individual amino acid sample of step (b) is mixed with the resin in a separate vessel.

50. The method of claim 47, wherein each individual amino acid sample of step (b) comprises a mixture comprising about 90% to 100% of an Fmoc-protected amino acid $HO_2C$-R'-NH-Fmoc and 0 to about 10% of corresponding acylated amino acid $HO_2C$-R'-NHCOR''.

51. The method of claim 50, wherein the acl (COR'') group is selected from the group consisting of $CH_3CO$-, butyryl- , PrCO-, benzoyl, formyl, 9-anthracenylcarbonyl and equivalents thereof.

52. The method of claim 47, wherein the mixing of step (c) is in a solvent is selected from the group consisting of DMF, NMP and equivalents thereof.

53. The method of claim 47, wherein the carbodiimide reagent of step (c) is selected from the group consisting of dicyclohexyldiimide, diisopropylcarbodiimide, and equivalents thereof.

54. The method of claim 47, wherein the base of step (c) is diisopropylethylamine, or equivalents thereof.

55. The method of claim 47, wherein mixing of step (c) takes place for between about 2 and about 24 hours at room temperature.

56. The method of claim 47, wherein the coupling reagent of step (c) is selected from the group consisting of PyBroP, HATU, PyBOP, HBTU and equivalents thereof.

57. The method of claim 47, further comprising capping the terminal amino group with a COR''' group by reaction with the corresponding anhydride $(R'''CO)_2O$ or halide R'''COX or equivalent.

58. The method of claim 57, wherein the COR''' capping group is selected from the group consisting of acetyl ($COCH_3$), benzoyl (COPh), formyl (COH), propionyl (COEt), 9-anthracenylcarbonyl, and equivalents.

59. The method of claim 47, wherein the amino acids are selected from the group consisting of L-Ala, D-Ala, L-Nva, D-Nva, L-Met, D-Met, L-Phe, D-Phe, L-Ser(tBu), D-Ser(tBu), L-Leu, D-Leu, L-Hfe, D-Hfe, L-Gin, D-Gin, L-Gln, D-Gln, L-Trp(Boc), D-Trp(Boc), cis 2-aminocyclohexane carboxylic acid, trans 2-aminocyclohexane carboxylic acid, cis 4-aminocyclohexane carboxylic acid, trans 4-aminocyclohexane carboxylic acid, aminomethylbenzoic acid, $HO_2C$-R'-NHFmoc where R' = $(CH_2)_n$ where n is an integer between 1 to about 12 and where R' = all positional isomers of biphenylmethyl ($C_6H_4$-$C_6H_4$-$CH_2$-).

60. The method of claim 47, wherein the peptide library comprises the general formula Resin-$(CH_2)_y$-$(C_6H_4)$-C(Ar)(Ph)-W-R-Z-(CO-R'-$NH)_n$-COR''', wherein each unit n is terminated by 0 to about 10% COR'', wherein COR'' is selected from the group consisting of $CH_3CO$-, butyryl- , PrCO-, benzoyl, formyl and equivalents thereof, and COR''' is selected from the group consisting of acetyl ($COCH_3$), benzoyl (COPh), formyl (COH), propionyl (COEt), 9-anthracenylcarbonyl and equivalents.

61. The method of claim 47, wherein the resin-conjugated peptide library is a resin-conjugated monoamide library.

62. The method of claim 61, wherein the resin-conjugated peptide library is a resin-conjugated di-peptide library.

63. The method of claim 62, wherein the resin-conjugated peptide library is a resin-conjugated tri-peptide library.

64. The method of claim 63, wherein the resin-conjugated peptide library is a resin-conjugated tetrapeptide library.

65. A resin-conjugated peptide library made by a method comprising the following steps:

(a) providing a spacer-conjugated triarylmethane resin, wherein the spacer comprises the general formula -W-R-ZH, where W and Z are selected from the group consisting of O, S, and NH, and R is an alkyl or an oxyalkyl spacer or equivalent or oligomeric version thereof;

(b) providing a plurality of 9-fluorenylmethoxycarbonyl (Fmoc) protected amino acid samples, wherein an amino acid comprises the general formula $HO_2C$-R'-NHFmoc;

(c) mixing each amino acid sample of step (b) with a spacer-conjugated triarylmethane resin of step (a) by a 9-fluorenylmethoxycarbonyl (Fmoc) procedure with a carbodiimide reagent, a hydroxybenzotriazole and a base, or, a coupling reagent and a base,

(d) releasing the terminal Fmoc group by stirring the resin of step (c) in an about 20 to 30% solution of piperidine, or equivalent, in a solvent selected from the group consisting of a dichloromethane, a NMP, a THF, a DMF and an equivalent, thereby making a deprotected resin;

(e) pooling the contents of all individual vessels, mixing, and redividing into equal amounts in the same number of samples as in step (b);

(f) repeat steps (c) to (e) until the peptide has the desired length, thereby making the resin-conjugated peptide library.

66. The resin-conjugated peptide library of claim 65, wherein the resin-conjugated peptide library is selected from the group consisting of a resin-conjugated monoamide library, a resin-conjugated di-peptide library, a resin-conjugated tri-peptide library, a resin-conjugated tetrapeptide library, a resin-

conjugated library wherein each member is five peptides in length, a resin-conjugated library wherein each member is six peptides in length, a resin-conjugated library wherein each member is seven peptides in length, a resin-conjugated library wherein each member is eight peptides in length, a resin-conjugated library wherein each member is nine peptides in length and a resin-conjugated library wherein each member is ten peptides in length.

67. A resin-conjugated peptide library comprising the general formula Resin-$(CH_2)_y$-$(C_6H_4)$-C(Ar)(Ph)-W-R-Z-(CO-R'-NH)$_n$-COR''', wherein each unit n is terminated by 0 to about 10% COR'', wherein COR'' is selected from the group consisting of $CH_3CO$-, butyryl- , PrCO-, benzoyl, formyl and equivalents thereof, and COR''' is selected from the group consisting of acetyl ($COCH_3$), benzoyl (COPh), formyl (COH), propionyl (COEt), 9-anthracenylcarbonyl and equivalents.

68. A method of making a resin-conjugated polyborane-amine adduct library comprising the following steps:

(a) providing a spacer-conjugated triarylmethane resin, wherein the spacer comprises the general formula -W-R-ZH, where W and Z are selected from the group consisting of O, S, and NH, and R is an alkyl or an oxyalkyl spacer or equivalent or oligomeric version thereof;

(b) providing a plurality of 9-fluorenylmethoxycarbonyl (Fmoc) protected amino acid samples, wherein an amino acid comprises the general formula $HO_2C$-R'-NHFmoc;

(c) mixing each amino acid sample of step (b) with a spacer-conjugated triarylmethane resin of step (a) by a 9-fluorenylmethoxycarbonyl (Fmoc) procedure with a carbodiimide reagent, a hydroxybenzotriazole and a base, or, a coupling reagent and a base,

(d) releasing the terminal Fmoc group by stirring the resin of step (c) in an about 20 to 30% solution of piperidine, or equivalent, in a solvent selected from the group consisting of a dichloromethane, a DMF, a NMP, a THF and equivalents, thereby making a deprotected resin;

(e) pooling the contents of all individual vessels, mixing, and redividing into equal amounts in the same number of samples as in step (b);

(f) repeat steps (c) to (e) until the peptide has the desired length, thereby making a resin-conjugated peptide library; and,

(g) reacting the resin-conjugated peptide library of step (f) with about 0.1 to about 1.0 M $BH_3$ or equivalent and THF or equivalent, thereby making a resin-conjugated polyborane-amine adduct library.

69. The method of claim 68, wherein the reaction of step (e) comprises a temperature of about 65°C for 12 hours to about 5 days.

70. A resin-conjugated polyborane-amine adduct library made by a method comprising the following steps:

(a) providing a spacer-conjugated triarylmethane resin, wherein the spacer comprises the general formula -W-R-ZH, where W and Z are selected from the group consisting of O, S, and NH, and R is an alkyl or an oxyalkyl spacer or equivalent or oligomeric version thereof;

(b) providing a plurality of 9-fluorenylmethoxycarbonyl (Fmoc) protected amino acid samples, wherein an amino acid comprises the general formula $HO_2C$-R'-NHFmoc;

(c) mixing each amino acid sample of step (b) with a spacer-conjugated triarylmethane resin of step (a) by a 9-fluorenylmethoxycarbonyl (Fmoc) procedure with a carbodiimide reagent, a hydroxybenzotriazole and a base, or, a coupling reagent and a base,

(d) releasing the terminal Fmoc group by stirring the resin of step (c) in an about 20 to 30% solution of piperidine, or equivalent, in a solvent selected from the group consisting of a dichloromethane, a DMF, a NMP, a THF and equivalents, thereby making a deprotected resin;

(e) pooling the contents of all individual vessels, mixing, and redividing into equal amounts in the same number of samples as in step (b);

(f) repeat steps (c) to (e) until the peptide has the desired length, thereby making the resin-conjugated peptide library; and,

(g) reacting the resin-conjugated peptide library of step (f) with about 0.1 to about 1.0 M $BH_3$ or equivalent and THF or equivalent, thereby making a resin-conjugated polyborane-amine adduct library.

71. A resin-conjugated polyborane-amine adduct library comprising a spacer-conjugated triarylmethane resin, wherein the spacer is selected

from the group consisting of $-HN[(CH_2)_nO(CH_2)_n]_m NH_2$, wherein n is an integer between 1 and about 5 and m is an integer between 1 and about 25.

72. A method of making a resin-conjugated polyamine library comprising the following steps:

(a) providing a spacer-conjugated triarylmethane resin, wherein the spacer comprises the general formula -W-R-ZH, where W and Z are selected from the group consisting of O, S, and NH, and R is an alkyl or an oxyalkyl spacer or equivalent or oligomeric version thereof;

(b) providing a plurality of 9-fluorenylmethoxycarbonyl (Fmoc) protected amino acid samples, wherein an amino acid comprises the general formula $HO_2C$-R'-NHFmoc;

(c) mixing each amino acid sample of step (b) with a spacer-conjugated triarylmethane resin of step (a) by a 9-fluorenylmethoxycarbonyl (Fmoc) procedure with a carbodiimide reagent, a hydroxybenzotriazole and a base, or, a coupling reagent and a base,

(d) releasing the terminal Fmoc group by stirring the resin of step (c) in an about 20 to 30% solution of piperidine, or equivalent, in a solvent selected from the group consisting of a dichloromethane, a DMF, a NMP, a THF and equivalents, thereby making a deprotected resin;

(e) pooling the contents of all individual vessels, mixing, and redividing into equal amounts in the same number of samples as in step (b);

(f) repeat steps (c) to (e) until the peptide has the desired length, thereby making the resin-conjugated peptide library;

(g) reacting the resin-conjugated peptide library of step (f) with about 0.1 to about 1.0 M $BH_3$ or equivalent and THF or equivalent, thereby making a resin-conjugated polyborane-amine adduct library; and,

(h) reacting the resin-conjugated polyborane-amine adduct library of step (g) under oxidative conditions, thereby making a resin-conjugated polyamine library.

73. The method of claim 72, wherein step (h) is replaced with the following alternative which comprises shaking the resin, after the borane reduction and the usual washings of step (g) in a solution of neat piperidine or equivalent at

65°C for 12-16 hours, filtering the resin at room temperature and washing it several times with a solvent selected from the group consisting of a dichloromethane, methanol, a DMF, a NMP, a THF and equivalents, thereby making deprotected resin.

74. The method of claim 72, wherein the oxidative conditions are mild oxidative conditions using excess iodine in THF.

75. The method of claim 72, wherein the mildly oxidative conditions comprise mildly acidic conditions at about pH 5.

76. The method of claim 75, wherein the mildly acidic conditions comprise buffering by an acetic acid-trialkylamine buffer in a 2:1 volume ratio in THF.

77. The method of claim 72, wherein the oxidative conditions of step (h) comprise a solution comprising $I_2$, AcOH, diisopropylethylamine (DIPEA), triethylamine, THF, or equivalents thereof and a reaction time of about 1 to about 6 hours.

78. The method of claim 72, further comprising reaction with a solution comprising an $Et_3N$, THF, DMF, or equivalents thereof.

79. A resin-conjugated polyamine library made by a method as claimed in claim 72 or 73, or an obvious equivalent thereof.

80. A resin-conjugated polyamine library comprising a spacer-conjugated triarylmethane resin, wherein the spacer is $-HN[(CH_2)_nO(CH_2)_n]_m NH_2$, wherein n is an integer between 1 and about 5 and m is an integer between 1 and about 25, and the polyamine library comprises the general formula Resin-$(CH_2)_y$-$(C_6H_4)$-C(Ar)(Ph)-W-R-Z-$(CH_2$-R'-$NH)_n$-$CH_2$R''', wherein each unit n is terminated by 0 to about 10% COR'', wherein COR'' is selected from the group consisting of $CH_3CO$-, butyryl- , PrCO-, benzoyl, formyl and equivalents thereof, and COR''' is selected from the group consisting of acetyl ($COCH_3$), benzoyl (COPh), formyl (COH), propionyl (COEt), 9-anthracenylcarbonyl and equivalents, wherein R is an alkyl substituent.

81. A method for screening for a polyamine binding molecule in a biological sample comprising the following steps:

(a) providing a biological sample;

(b) providing a resin-conjugated polyamine library as set forth in claim 78 or claim 79;

(c) mixing the biological sample with the resin-conjugated polyamine library; and

(d) washing the resin-conjugated polyamine library and determining if a biological molecule has specifically bound to a resin-conjugated polyamine.

82. The method of claim 81, wherein the biological molecule comprises a nucleic acid.

83. The method of claim 82, wherein the nucleic acid comprises a DNA.

84. The method of claim 82, wherein the nucleic acid comprises an RNA.

85. The method of claim 81, wherein the biological molecule comprises a lipid.

86. The method of claim 81, wherein the biological molecule comprises a polypeptide or a protein.

87. The method of claim 81, wherein the biological molecule comprises a polysaccharide.

88. The method of claim 81, wherein the biological sample is mixed with the resin-conjugated polyamine library under conditions comprising buffered water.

89. A method for screening for a polyamine binding molecule in a biological sample comprising the following steps:

(a) providing a biological sample;

(b) providing a resin-conjugated polyamine library as set forth in claim 78 or claim 79, wherein the polyamine library is further derivatized to a tertiary polyamine library by alkylation with an alkyl or an aryl halide or further derivatized by reductive amination with aldehydes and a hydride reagent, or, further derivatized to an acylpolyamine library by reaction with acid anhydrides having the general formula $(R''''CO)_2O$ or halides having the general formula $R''''COX$ or $R''''COCl$ or $R''''CO_2H$ or equivalents, wherein R is an alkyl or an aryl substituent, X is a halogen;

(c) mixing the biological sample with the resin-conjugated polyamine library; and

(d) washing the resin-conjugated polyamine library and determining if a biological molecule has specifically bound to a resin-conjugated polyamine.

90. The method of claim 89, wherein the alkyl halide comprises a compound selected from the group consisting of ortho-bromomethylboronic acid anhydride or a corresponding boronic ester, allylbromide, benzyl bromide, methyl iodide, ethyl iodide, and equivalents thereof.

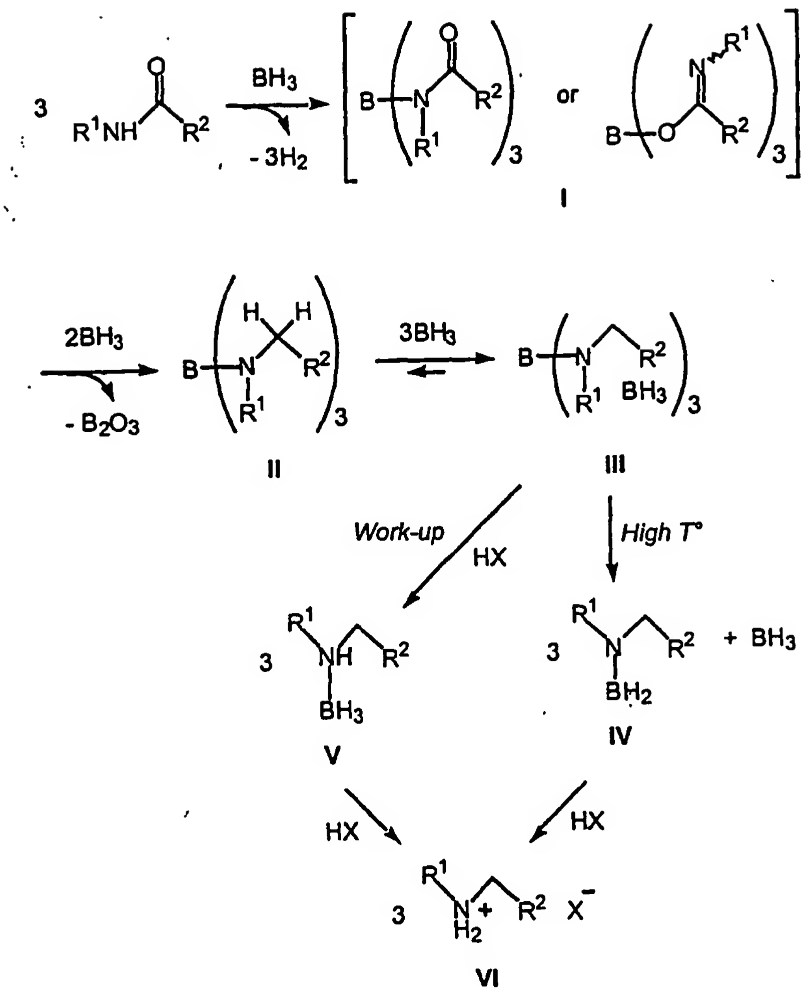
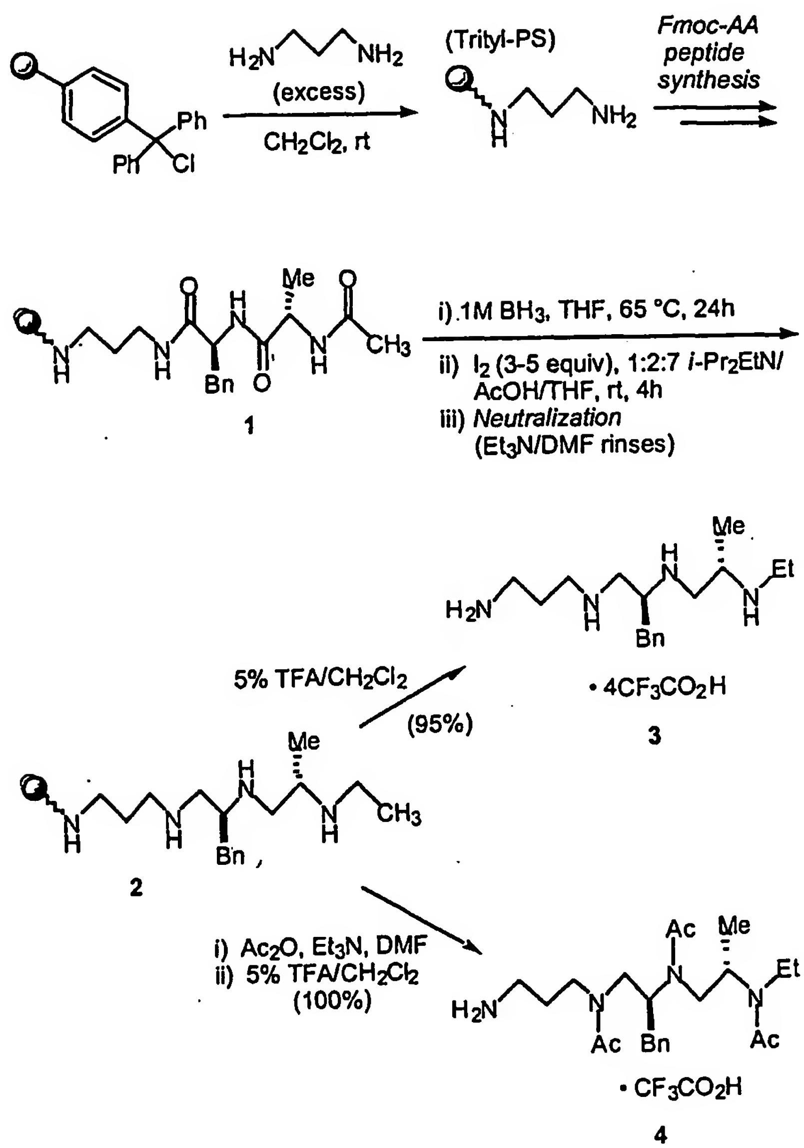
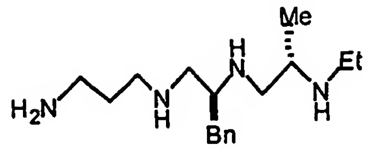

3
BH3
- 3H2
or
I
2BH3
- B2O3
II
3BH3
III
Work-up
HX
High T°
+ BH3
V
IV
HX
HX
X
VI

FIG. 1



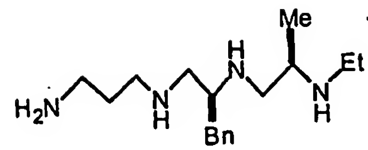
H2N NH2
(excess)
CH2Cl2, rt
Ph
Ph Cl
(Trityl-PS)
NH2
Fmoc-AA
peptide
synthesis
Me
CH3
Bn
1
i) 1M BH3, THF, 65 °C, 24h
ii) I2 (3-5 equiv), 1:2:7 i-Pr2EtN/
AcOH/THF, rt, 4h
iii) Neutralization
(Et3N/DMF rinses)
5% TFA/CH2Cl2
(95%)
H2N
Et
• 4CF3CO2H
3
2
i) Ac2O, Et3N, DMF
ii) 5% TFA/CH2Cl2
(100%)
Ac
• CF3CO2H
4


FIG. 2



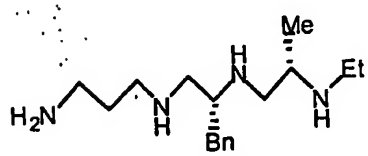
• $4CF_3CO_2H$

LL-3



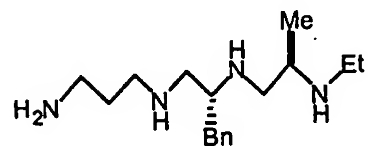
• $4CF_3CO_2H$

LD-3



• $4CF_3CO_2H$

DL-3



• $4CF_3CO_2H$

DD-3

FIG. 3

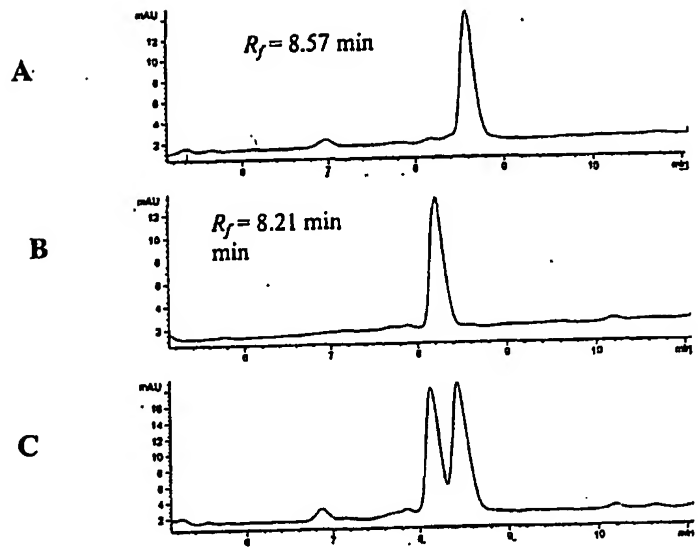



FIG. 4

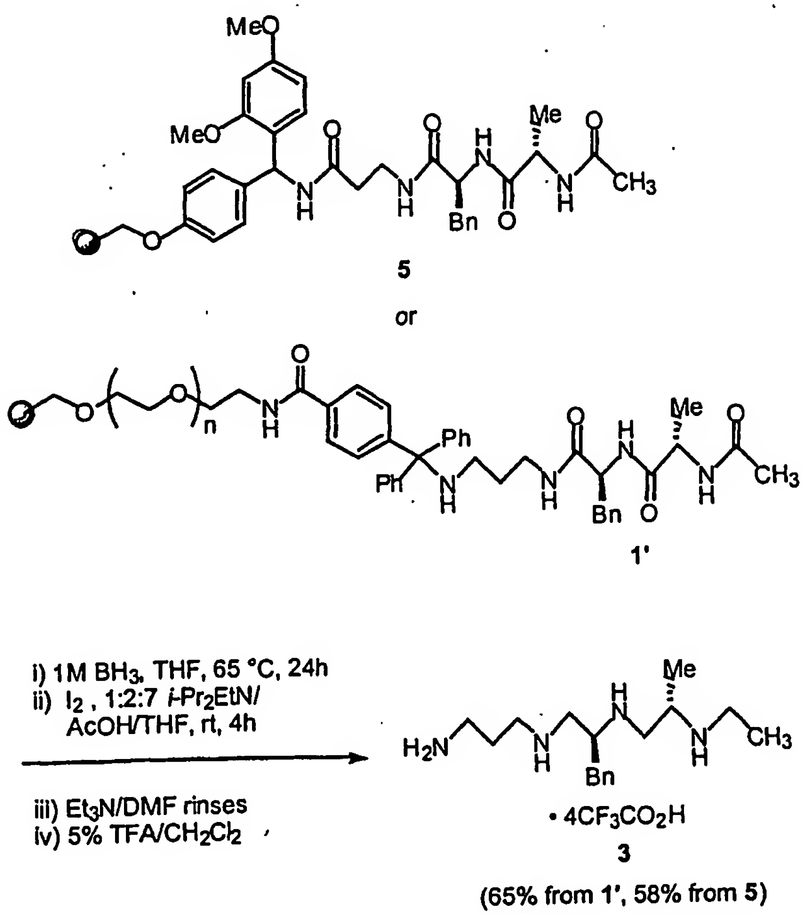
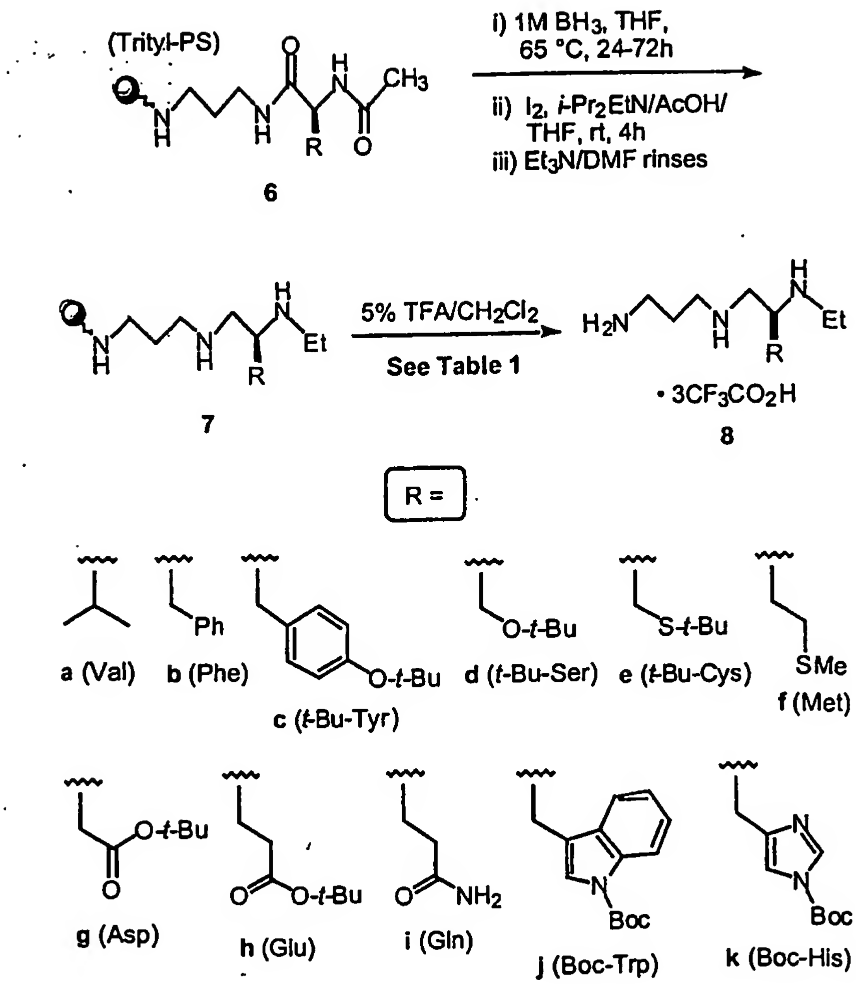

MeO
MeO
Me
CH3
Bn
5
or
n
Ph
Ph
Me
CH3
Bn
1'
i) 1M BH3, THF, 65 °C, 24h
ii) I2 , 1:2:7 i-Pr2EtN/ AcOH/THF, rt, 4h
iii) Et3N/DMF rinses
iv) 5% TFA/CH2Cl2
Me
H2N
CH3
Bn
• 4CF3CO2H
3
(65% from 1', 58% from 5)

FIG. 5



(Trityl-PS)
6
i) 1M BH3, THF, 65 °C, 24-72h
ii) I2, i-Pr2EtN/AcOH/ THF, rt, 4h
iii) Et3N/DMF rinses
7
5% TFA/CH2Cl2
See Table 1
• 3CF3CO2H
8
R =
a (Val)
b (Phe)
c (t-Bu-Tyr)
d (t-Bu-Ser)
e (t-Bu-Cys)
f (Met)
g (Asp)
h (Glu)
i (Gln)
j (Boc-Trp)
k (Boc-His)


FIG. 6

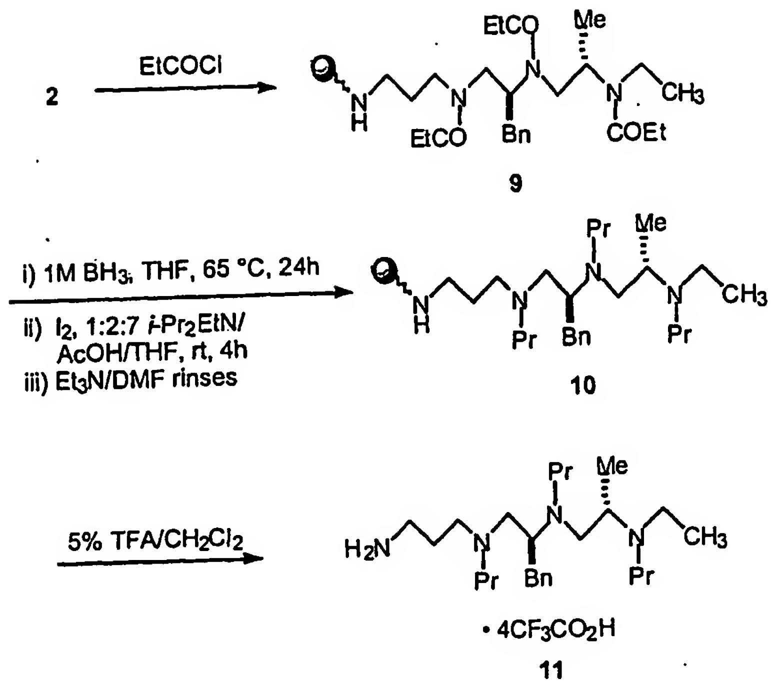

2
EtCOCl
EtCO
Me
N
H
EtCO
Bn
COEt
CH3
9
i) 1M BH3, THF, 65 °C, 24h
ii) I2, 1:2:7 i-Pr2EtN/ AcOH/THF, rt, 4h
iii) Et3N/DMF rinses
Pr
Me
Bn
CH3
10
5% TFA/CH2Cl2
H2N
Pr
Me
Bn
CH3
• 4CF3CO2H
11

FIG. 7

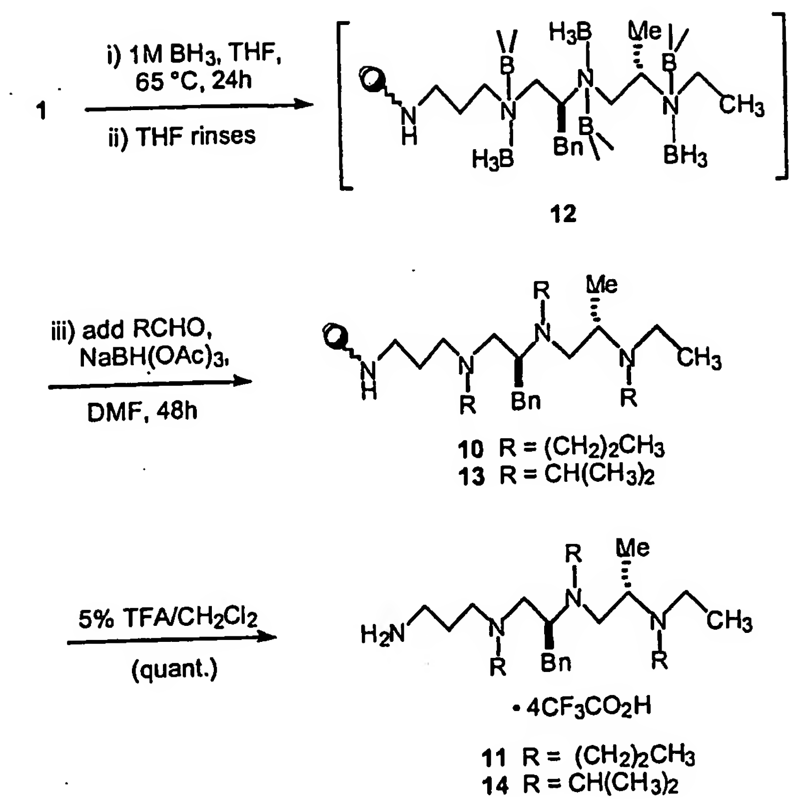

1
i) 1M BH3, THF, 65 °C, 24h
ii) THF rinses
H3B
Me
CH3
Bn
BH3
12
iii) add RCHO, NaBH(OAc)3,
DMF, 48h
R
Bn
10 R = (CH2)2CH3
13 R = CH(CH3)2
5% TFA/CH2Cl2
(quant.)
H2N
• 4CF3CO2H
11 R = (CH2)2CH3
14 R = CH(CH3)2

FIG. 8

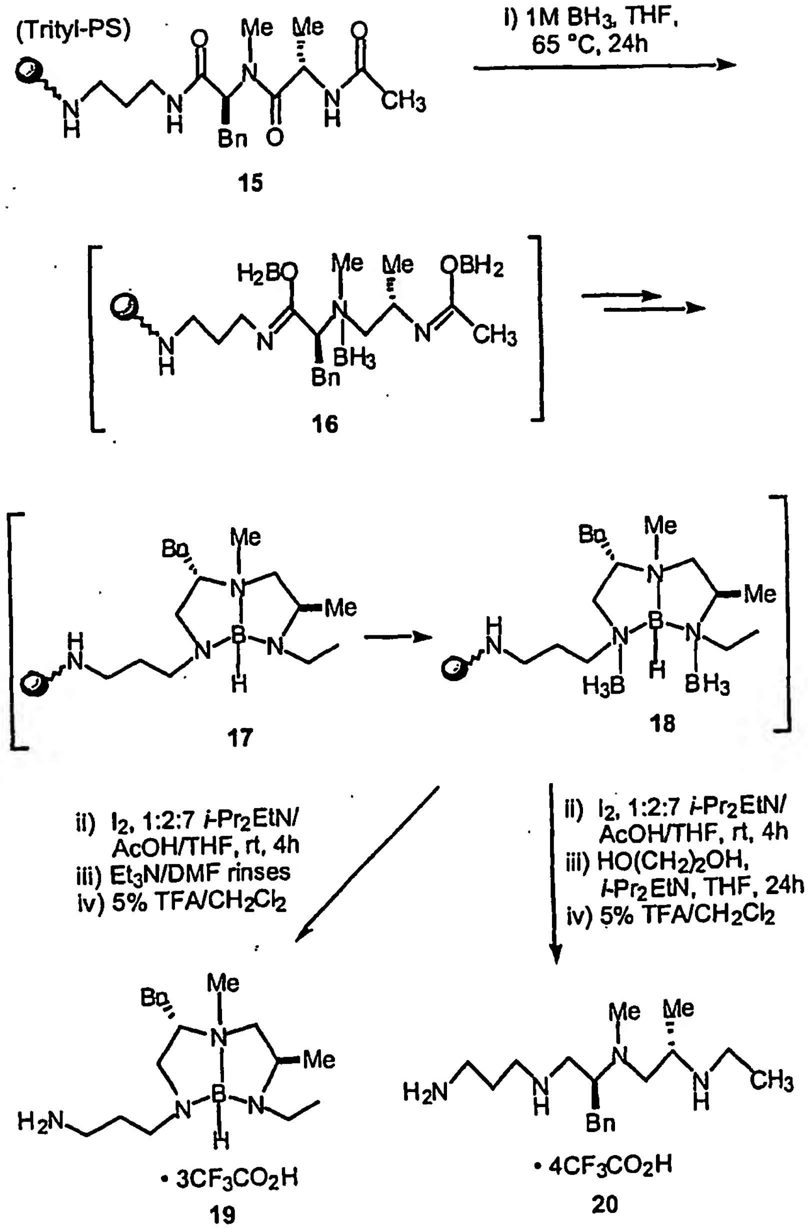
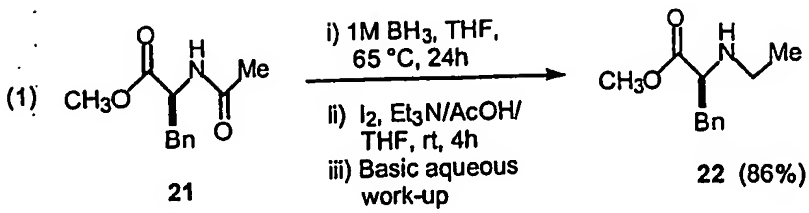
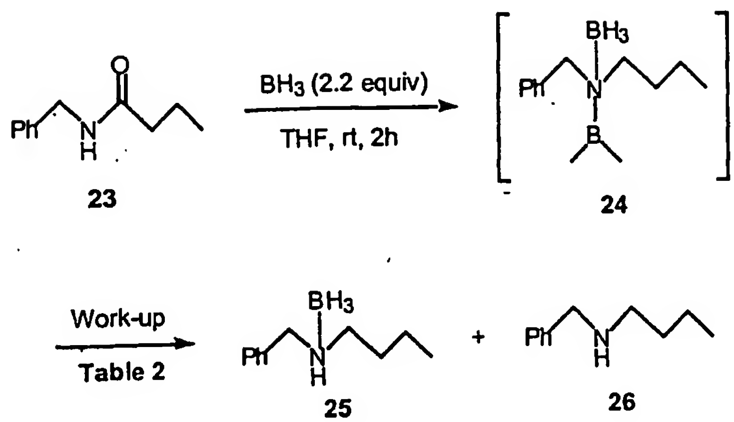

(Trityl-PS)
i) 1M $BH_3$, THF, 65 °C, 24h
15
16
17
18
ii) $I_2$, 1:2:7 *i*-$Pr_2$EtN/ AcOH/THF, rt, 4h
iii) $Et_3$N/DMF rinses
iv) 5% TFA/$CH_2Cl_2$
ii) $I_2$, 1:2:7 *i*-$Pr_2$EtN/ AcOH/THF, rt, 4h
iii) $HO(CH_2)_2OH$, *i*-$Pr_2$EtN, THF, 24h
iv) 5% TFA/$CH_2Cl_2$
• $3CF_3CO_2H$
19
• $4CF_3CO_2H$
20

FIG. 9



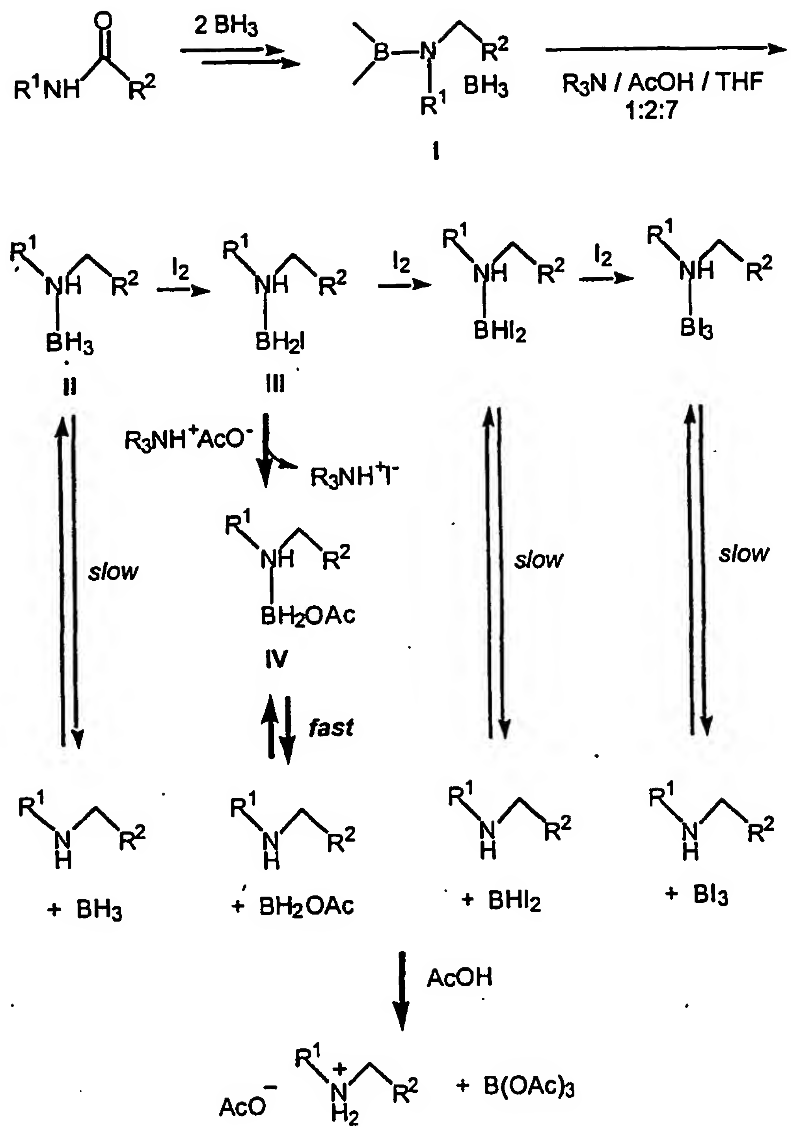

(2) $R_3N{-}BH_3 + I_2 + 3H_2O \longrightarrow R_3NH^+ + H_3BO_3 + 6I^- + 5H^+$

FIG. 10



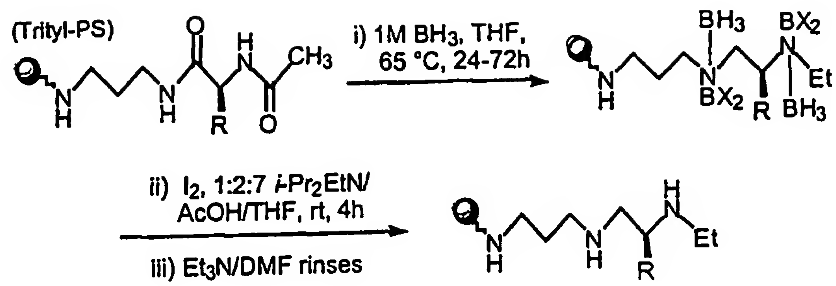
Ph
N
H
O
23
BH3 (2.2 equiv)
THF, rt, 2h
BH3
Ph
N
B
24
Work-up
Table 2
BH3
Ph
N
H
25
+
Ph
N
H
26


FIG. 11



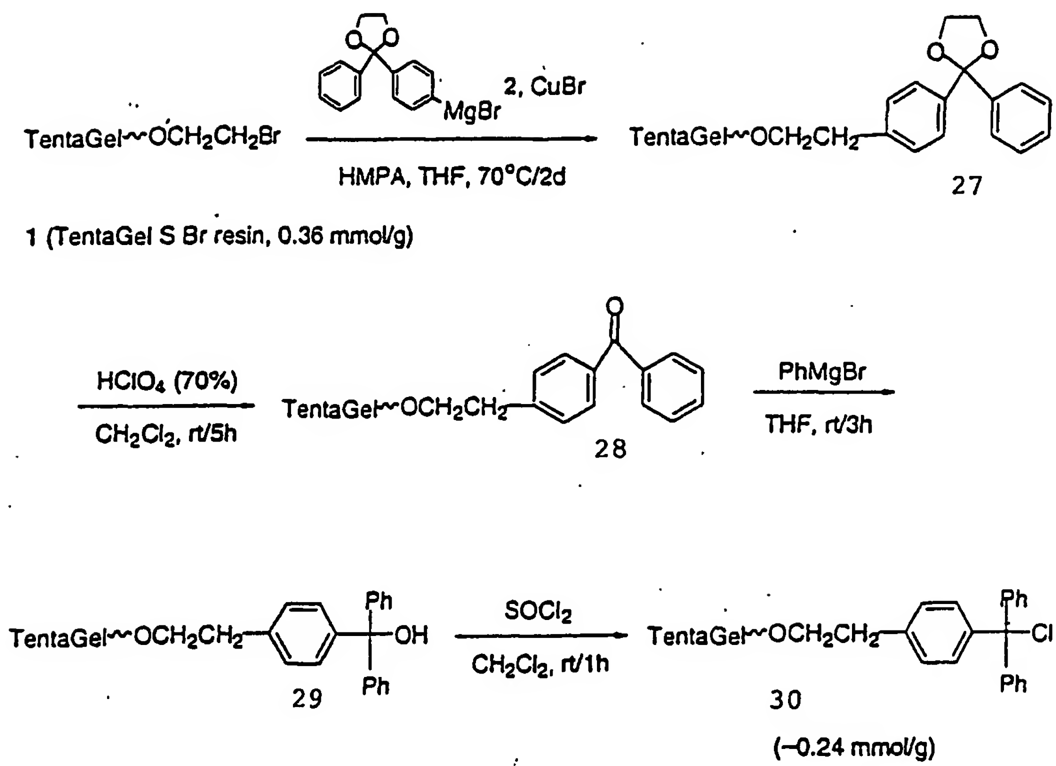
2 BH3
R1NH
R2
B
N
R2
BH3
R1
I
R3N / AcOH / THF
1:2:7
R1
NH
R2
BH3
II
I2
BH2I
III
I2
BHI2
I2
BI3
R3NH+AcO-
R3NH+I-
slow
BH2OAc
IV
fast
slow
slow
+ BH3
+ BH2OAc
+ BHI2
+ BI3
AcOH
AcO-
H2
+ B(OAc)3


FIG. 12



(Trityl-PS)
i) 1M BH3, THF, 65 °C, 24-72h
ii) I2, 1:2:7 i-Pr2EtN/ AcOH/THF, rt, 4h
iii) Et3N/DMF rinses


FIG. 13



2, CuBr
MgBr
TentaGel~OCH2CH2Br
HMPA, THF, 70°C/2d
TentaGel~OCH2CH2
27
1 (TentaGel S Br resin, 0.36 mmol/g)
HClO4 (70%)
CH2Cl2, rt/5h
TentaGel~OCH2CH2
28
PhMgBr
THF, rt/3h
TentaGel~OCH2CH2
Ph
OH
Ph
29
SOCl2
CH2Cl2, rt/1h
TentaGel~OCH2CH2
Ph
Cl
Ph
30
(~0.24 mmol/g)


FIG. 14

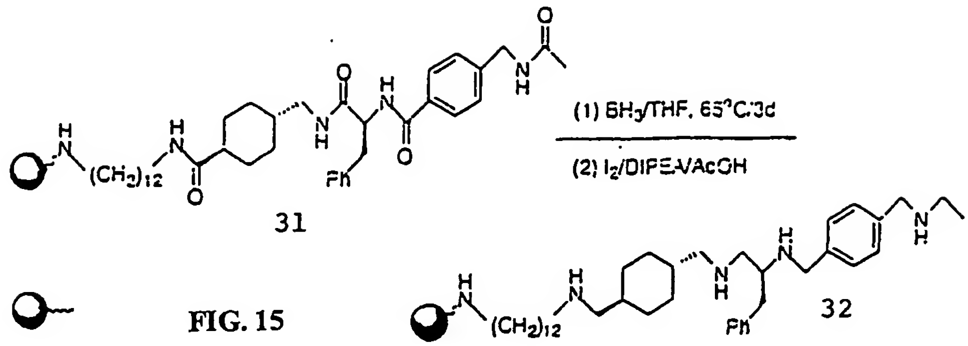



FIG. 15A

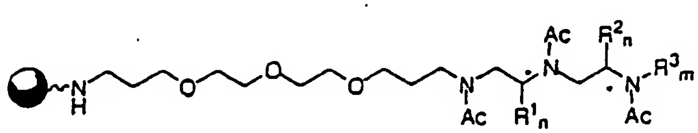
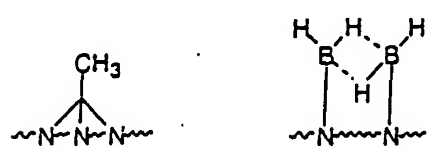


FIG. 15B



Structure I

Structure II

FIG. 15C

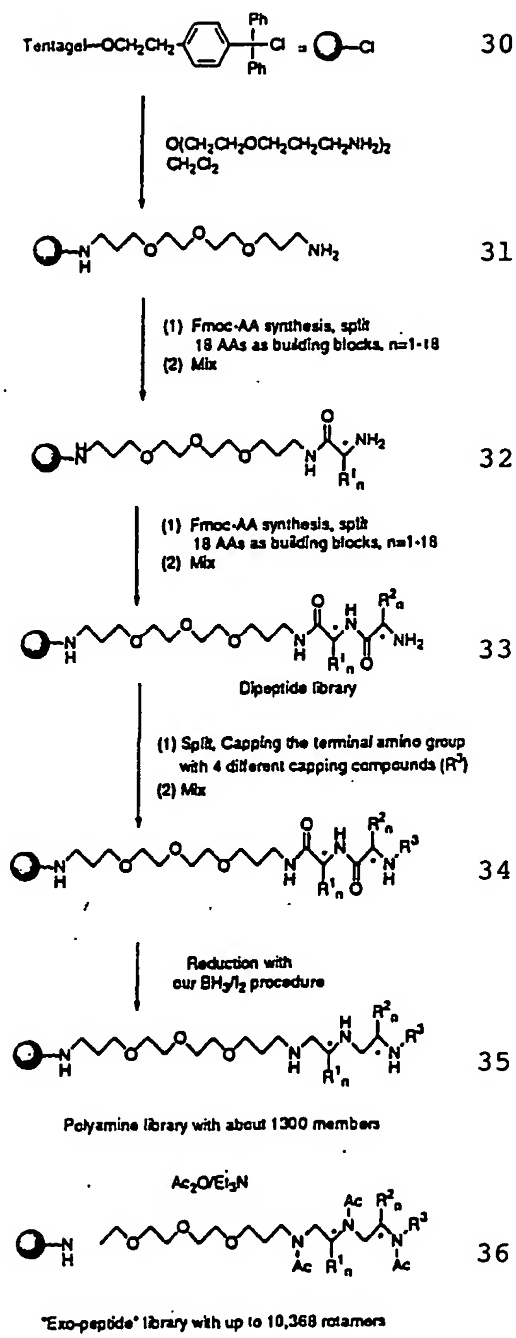

Tentagel—OCH2CH2
Ph
Cl
30
O(CH2CH2OCH2CH2CH2NH2)2
CH2Cl2
31
(1) Fmoc-AA synthesis, split
18 AAs as building blocks, n=1-18
(2) Mix
32
(1) Fmoc-AA synthesis, split
18 AAs as building blocks, n=1-18
(2) Mix
33
Dipeptide library
(1) Split, Capping the terminal amino group
with 4 different capping compounds (R3)
(2) Mix
34
Reduction with
our BH3/I2 procedure
35
Polyamine library with about 1300 members
Ac2O/Et3N
36
"Exo-peptide" library with up to 10,368 rotamers

FIG. 16

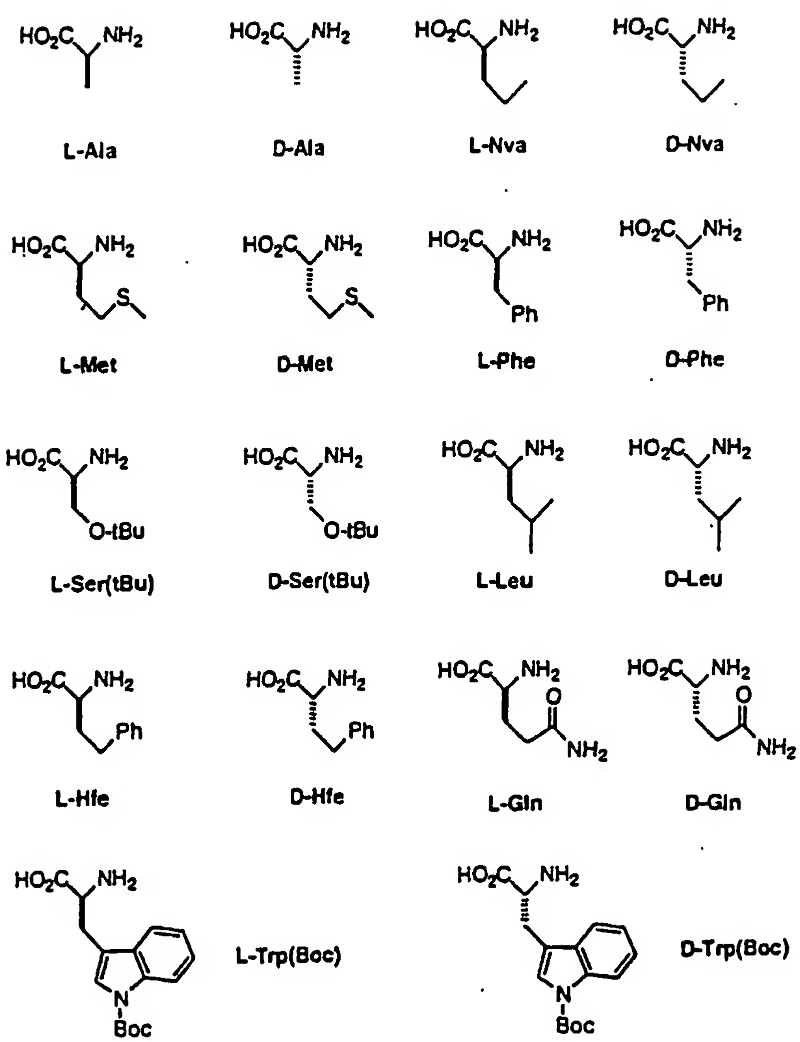

L-Ala
D-Ala
L-Nva
D-Nva
L-Met
D-Met
L-Phe
D-Phe
L-Ser(tBu)
D-Ser(tBu)
L-Leu
D-Leu
L-Hfe
D-Hfe
L-Gln
D-Gln
L-Trp(Boc)
D-Trp(Boc)

FIG. 17

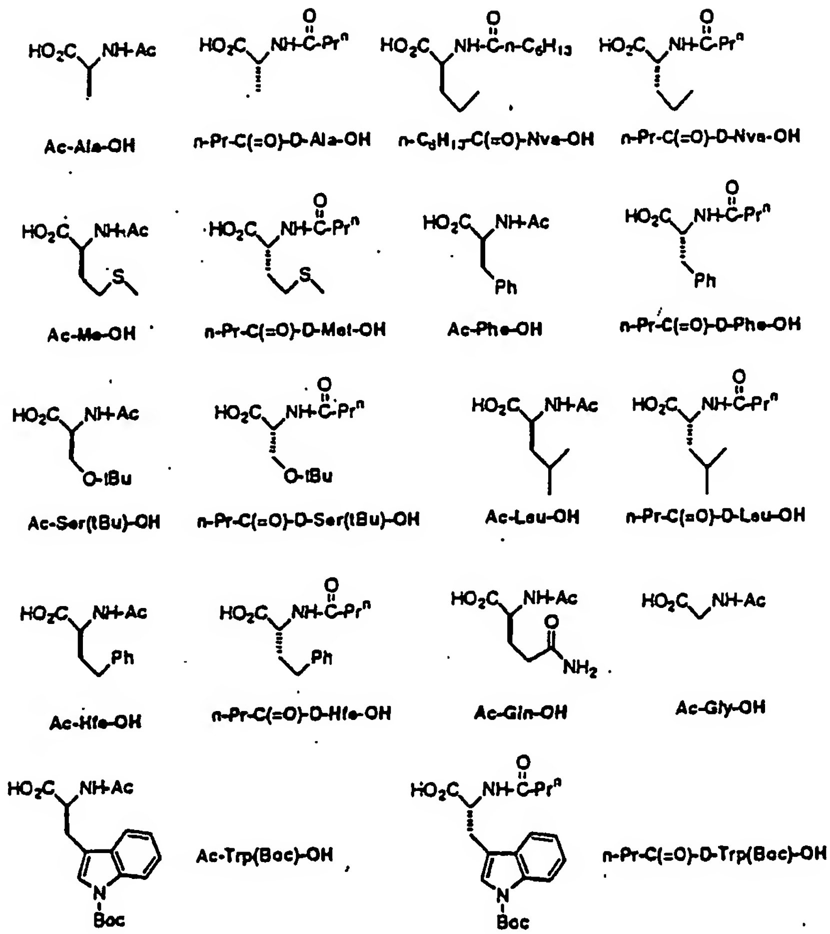

Ac-Ala-OH
n-Pr-C(=O)-D-Ala-OH
n-C6H13-C(=O)-Nva-OH
n-Pr-C(=O)-D-Nva-OH
Ac-Met-OH
n-Pr-C(=O)-D-Met-OH
Ac-Phe-OH
n-Pr-C(=O)-D-Phe-OH
Ac-Ser(tBu)-OH
n-Pr-C(=O)-D-Ser(tBu)-OH
Ac-Leu-OH
n-Pr-C(=O)-D-Leu-OH
Ac-Hfe-OH
n-Pr-C(=O)-D-Hfe-OH
Ac-Gln-OH
Ac-Gly-OH
Ac-Trp(Boc)-OH
n-Pr-C(=O)-D-Trp(Boc)-OH

FIG. 18

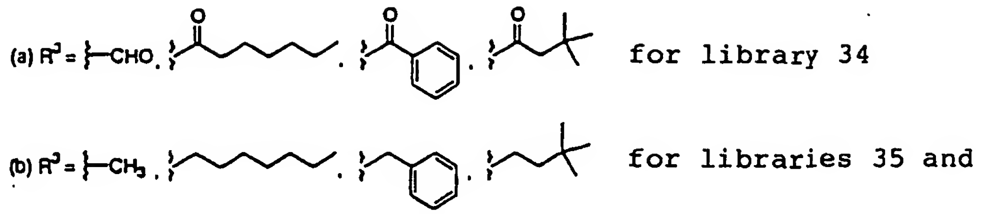



FIG. 19

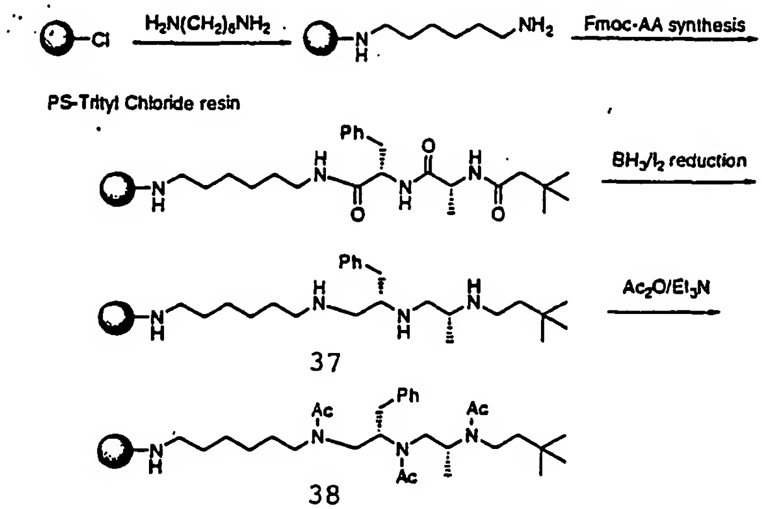



FIG. 20

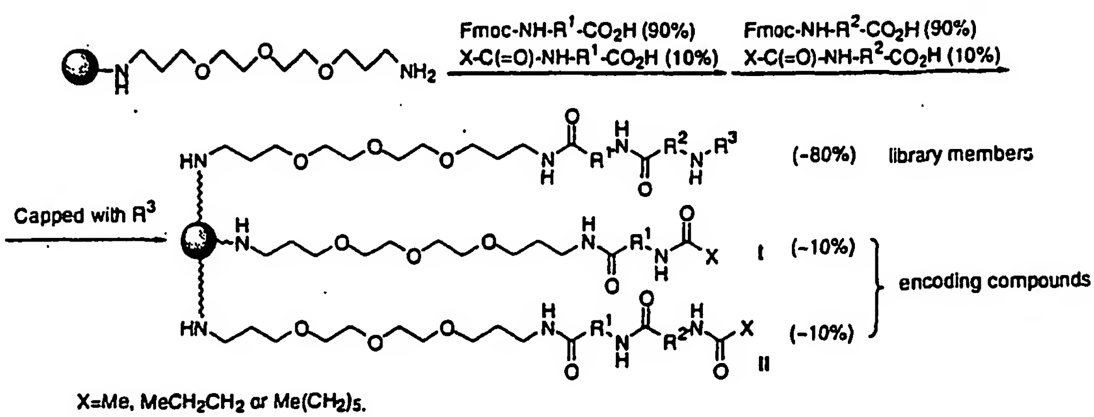



FIG. 21

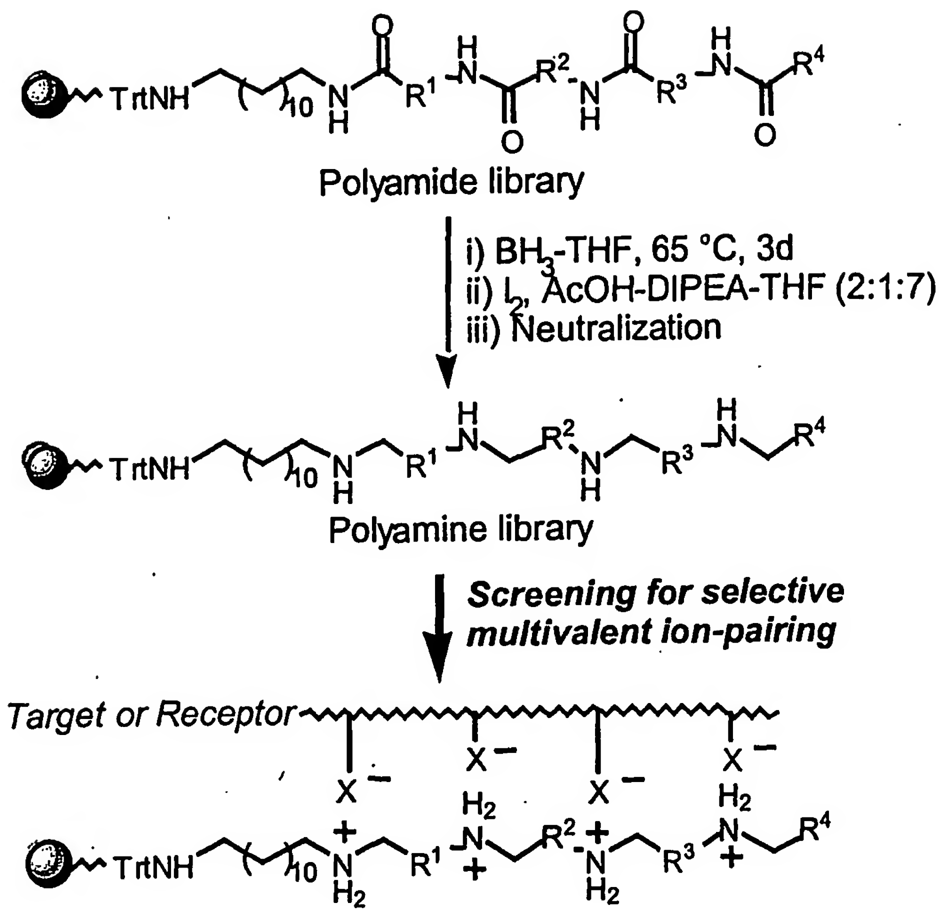
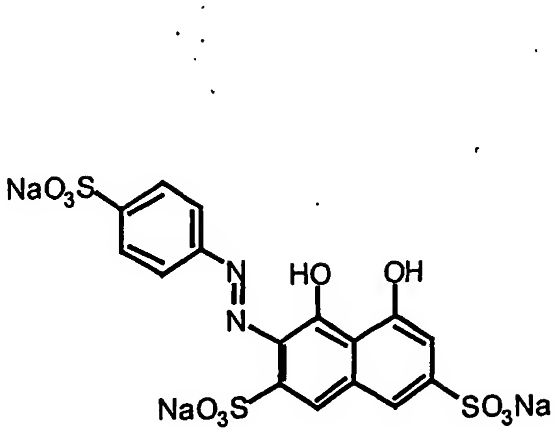

TrtNH
10
R1
R2
R3
R4
Polyamide library
i) BH3-THF, 65 °C, 3d
ii) I2, AcOH-DIPEA-THF (2:1:7)
iii) Neutralization
Polyamine library
Screening for selective multivalent ion-pairing
Target or Receptor

FIG. 22



1 *SPADNS* 2 *New Coccine*

FIG. 23

$HO_2C(CH_2)_{11}NH_2$

12Ado

$HO_2C(CH_2)_7NH_2$

8Aoc

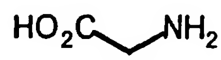
$HO_2C(CH_2)_5NH_2$

6Ahx

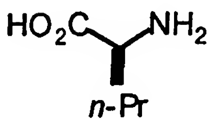
$HO_2C(CH_2)_3NH_2$

γAbu

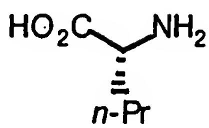
βAla



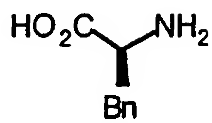
Gly



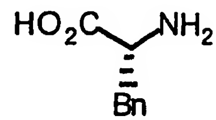
LNva



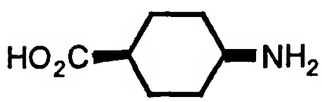
DNva



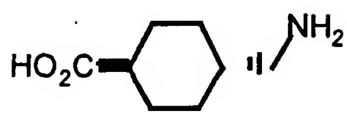
LPhe



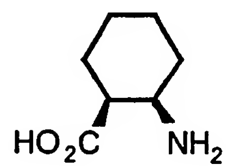
DPhe



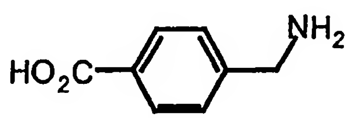
4Acc



4Amc



2Acc



4Amb

FIG. 24

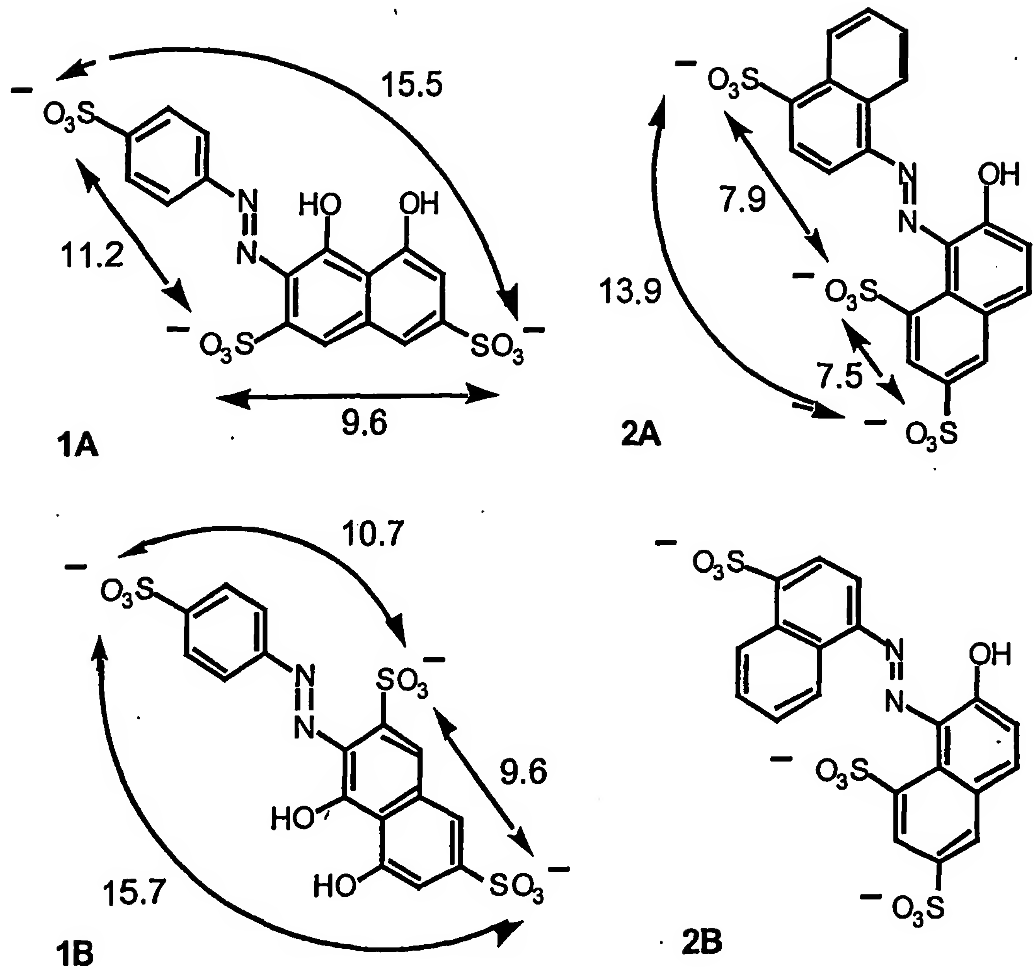

15.5
$^-O_3S$
11.2
N
N
HO
OH
$^-O_3S$
$SO_3^-$
9.6
1A
$^-O_3S$
7.9
N
N
OH
13.9
$^-O_3S$
7.5
$^-O_3S$
2A
10.7
$^-O_3S$
N
N
$SO_3^-$
9.6
HO
HO
$SO_3^-$
15.7
1B
$^-O_3S$
N
N
OH
$^-O_3S$
$^-O_3S$
2B

FIG. 25

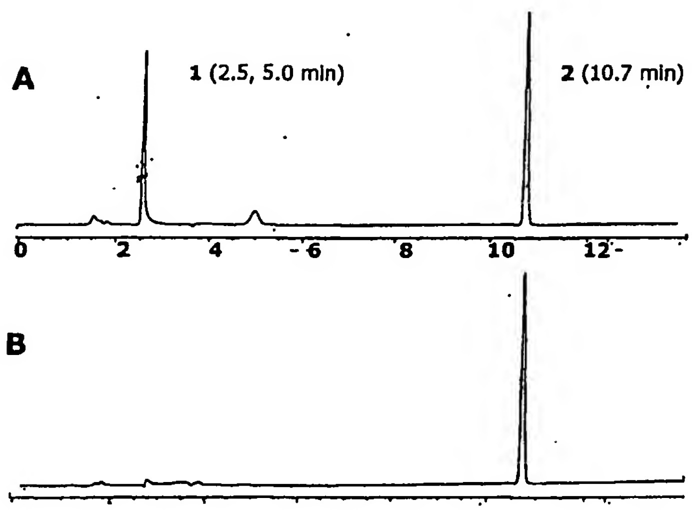



FIG. 26

# INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 02/00514

**A. CLASSIFICATION OF SUBJECT MATTER**
IPC 7 C08F8/00 G01N33/53

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X,Y | MANKU S ET AL: "A mild and general solid-phase method for the synthesis of chiral polyamines. Solution studies on the cleavage of borane-amine intermediates from the reduction of secondary amides" JOURNAL OF ORGANIC CHEMISTRY, vol. 66, no. 3, 9 February 2001 (2001-02-09), pages 874-885, XP002211756 the whole document --- | 1-90 |

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

30 August 2002

Date of mailing of the international search report

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL – 2280 HV Rijswijk
Tel. (+31–70) 340–2040, Tx. 31 651 epo nl,
Fax: (+31–70) 340–3016

Authorized officer

Allard, M

1

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication,where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X,Y | WANG F ET AL: "Solid phase syntheses of polyamine toxins HO-416b and PhTX-433. Use of an efficient polyamide reduction strategy that facilitates access to branched analogues"<br>ORGANIC LETTERS,<br>vol. 2, no. 11, 1 June 2000 (2000-06-01), pages 1581-1583, XP002211757<br>the whole document<br>--- | 1-90 |
| P,X,Y | MANKU S ET AL: "Combinatorial approach to selective multivalent ion pairing in mixed aqueous-organic media using bead-supported libraries of unnatural polyamines"<br>ORGANIC LETTERS,<br>vol. 4, no. 1,<br>10 January 2002 (2002-01-10), pages 31-34, XP002211758<br>the whole document<br>--- | 1-90 |
| Y | US 5 563 220 A (WEBBER R ET AL)<br>8 October 1996 (1996-10-08)<br>the whole document<br>--- | 1-90 |
| Y | EP 0 285 562 A (CIBA-GEIGY AG)<br>5 October 1988 (1988-10-05)<br>the whole document<br>--- | 1-90 |
| Y | DE 43 06 839 A (BAYER E)<br>8 September 1994 (1994-09-08)<br>the whole document<br>--- | 1-90 |
| Y | MARSH I R ET AL: "Synthetic methods for polyamine linkers and their application to combinatorial chemistry"<br>MOLECULAR DIVERSITY,<br>vol. 2, 1996, pages 165-170, XP000945040<br>the whole document<br>---- | 1-90 |

1

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/CA 02/00514

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
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| | | AU 2238992 A | 12-01-1993 |
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| | | EP 0543986 A1 | 02-06-1993 |
| | | JP 2722392 B2 | 04-03-1998 |
| | | JP 6502679 T | 24-03-1994 |
| | | WO 9222591 A1 | 23-12-1992 |
| EP 285562 A | 05-10-1988 | AT 88726 T | 15-05-1993 |
| | | AU 615181 B2 | 26-09-1991 |
| | | AU 1381488 A | 29-09-1988 |
| | | CA 1318462 A1 | 25-05-1993 |
| | | CA 1322008 A2 | 07-09-1993 |
| | | DD 274033 A5 | 06-12-1989 |
| | | DD 284031 A5 | 31-10-1990 |
| | | DD 296087 A5 | 21-11-1991 |
| | | DE 3880541 D1 | 03-06-1993 |
| | | DK 173088 A | 01-10-1988 |
| | | EP 0285562 A2 | 05-10-1988 |
| | | ES 2054863 T3 | 16-08-1994 |
| | | FI 881451 A ,B, | 01-10-1988 |
| | | GR 3007996 T3 | 31-08-1993 |
| | | HU 46713 A2 | 28-11-1988 |
| | | IE 61485 B | 02-11-1994 |
| | | IL 85884 A | 15-01-1992 |
| | | JP 2513775 B2 | 03-07-1996 |
| | | JP 63260946 A | 27-10-1988 |
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